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PROVIDENCE, RHODE ISLAND

CONTRIBUTIONS

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PREFACE

The papers which are collected in this fourth volume of Contributions have been written by officers or students in the Department of Comparative Anatomy of Brown University, and have recently appeared in various scientific journals. In the Table of Contents and on the title page of each paper will be found the place and time of publication.

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RECENT DEBTS TO BIOLOGY.

By F. P. GORHAM.

Providence Medical Journal, Vol. vi, No. 2, p. 49, March, 1905.

RECENT DEBTS TO BIOLOGY.*

By FREDERICK P. GORHAM, A. M.,
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I wish to speak to you this evening of some of the recent debts which we owe to biology, not at all as a justification of biology and biological work, but merely to call your attention to the widespread application to human interests which biological work is just now attaining. Biology itself needs no justification. The very subject matter of which it treats is of vital, lively interest to each one of us. A science of recent growth, whose very name has come but recently to the mouths of many of us, has already won for itself a place among sciences. Including as it does anatomy, physiology, pathology, anthropology, as well as zoology and botany, it comes very close to us on all sides, in all our activities, in health, in disease, in every branch of human endeavor in which we come in contact with that wonderful property of matter which we call life.

I know it is the contention of many workers in pure science that we ought not to look to the practical bearing of our research. It is true every newly discovered fact adds so much to the sum total of human knowledge, and the pursuit of truth for its own sake is its own reward. And further we never know when, nor in what way, a newly discovered fact may have its practical application. It sometimes requires years before we learn the value of a fact. I hope to show you before long that biology in particular gives us many instances of the practical application of knowledge sought out and developed for its own sake, with no consideration of its practical value.

But, on the other hand, we must beware the state of mind which disdains everything in science which has not an immediate application. Pasteur thus spoke of persons of this mind: "You know Franklin's saying? He was witnessing the first demonstration of a purely scientific discovery, and people around him said: 'but what is the use of it?' Franklin answered them, 'What is the use of a new born child?' Yes, what is the use of a new born child? * * * In your baby boys, fragile beings as they are, there are incipient magistrates, scientists and heroes; * * * a scientific discovery has but the merit of its existence; it awakens hope, and that is all. But let it be cultivated, let it grow, and you will see what it will become. Do you know when it first saw the light, this electric telegraph, one of the most marvelous applications of modern science? It was that memorable year 1822; Oersted, a Danish physicist held in his hand a piece of copper wire, joined by its extremities to the two poles of a Volta pile. On his table was a magnetized needle on its pivot, and he suddenly saw (by chance you will say, but chance only favors the mind which is prepared) the needle move and take up a position quite different from the one assigned to it by terrestrial magnetism;—that was the birth of the modern telegraph. Franklin's interlocutor might well have said when the needle moved, 'But what is the use of that?' "

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*A Faculty Lecture delivered November 30, 1904.

So on the one hand we have those who honor truth for its own sake, and on the other those who honor it for its application. There is a course midway between these two extremes, which recognizes the value of both opinions, and in a high degree biology occupies this middle ground, eminently practical on one side, yet thoroughly scientific, disinterested, seeking only after truth, on the other.

And so I might speak to you of the value of biology from the standpoint of pure science, its value as a field for research, its value as a teaching science, to develop the scientific instinct in the untrained mind; or I might explain to you how closely biological work touches the borderland of religion, bearing wrapped up in itself the origin of life, the problem of life, here and hereafter, and perhaps also something of our relation to spirit and to God. But it is not a theoretical problem which I wish to present to you, but some bare facts, some practical results of the application of biological work to every day problems.

As an introduction to the first application of biological work which I wish to discuss I must say a word or two about the work of Louis Pasteur, one of the greatest of biological workers, and one who appreciated as few others did, the value of every bit of human knowledge.

Pasteur began his scientific work as a chemist, and employed his time in elucidating the mysteries of tartaric acid. It was but a step from his study of this and related acids to a study of fermentation and the organisms, which by their growth in sugar solutions, wine and beer, bring about the production of these acids. From a study of these microscopic forms of plant life he moved on to the question of spontaneous generation, a question demanding a large share of the attention of the scientific men of his day, and one which of course had very little if any practical application. By his work he once and for all settled the question of spontaneous generation. He was able to say, "There is no circumstance known in which it can be affirmed that microscopic beings came into the world without germs, without parents similar to themselves. Those who affirm it have been duped by illusions, by ill conducted experiments, spoiled by errors that they either did not perceive or did not know how to avoid." Never will the doctrine of spontaneous generation recover from the blow of his simple experiments.

It was but another step for this master of research to begin a study of the diseases of wine and beer, faulty fermentations. And it was not long before he declared "that the alterations of wines are coexistent with the presence and multiplication of microscopic vegetations." It was from this knowledge that he was able to devise methods of prevention, and the wine industry of France owes untold millions to the work of this one man.

But still his work was not done. At about this time, 1865–1870, an epidemic was ruining in terrible proportions the industry of the cultivation of silkworms, another of the great industries of France. Every hypothesis as to cause was suggested, books and treatises with suggestions for prevention abounded, but in vain. The authorities appealed to Pasteur to undertake a study of the disease and to suggest means of prevention. He had become master of the diseases of wine and beer, why not of the silkworm? It was not long and the victory was his. Not only the cause but the means of prevention was proclaimed, and Pasteur was again the saviour of an industry the value of which to his country it would be difficult to estimate.

From germs in silkworms to germs in human beings was another natural step. Lister, the celebrated English surgeon, "having," he said "meditated on Pasteur's theory of germs and proclaimed himself his follower," convinced that complications and infections of wounds were caused by their giving access to living organisms and infectious germs, elements of trouble and often of death, had already in 1867 inaugurated a method of treatment. In one word he instituted antisepsis, without which all our modern surgery, with its astonishing results, its cure and prevention of disease, deformity and pain, would be impossible.

For a long time Pasteur, also, had meditated on the cause of human diseases. He had directed his attention primarily to a study of hydrophobia. He found that in animals which die of this disease, the salivary glands, the pancreas and the nervous system, contain the infection, and that the disease can be produced by an inoculation of small pieces of these organs. He also observed that the virulence of the poison was less in animals that had been dead for some time than in those just killed. A method of attenuating the virulence was thus suggested to Pasteur, and the idea of using it as a protective vaccination soon followed. The genius of Pasteur did not cease with the production of immunity, but, we rejoice to add, extended to the kindred subject of therapy and gave us a cure for hydrophobia. And now both in this country and in Europe we have Pasteur Institutes, devoted to the treatment of hydrophobia, and the large number of lives saved annually is a most fitting monument to the perseverance and scientific attainment of the man for whom they are named.

Pasteur's suggestions and successful results in the case of hydrophobia have since been extended to other diseases, and it is remarkable to note that all of his results, so carefully did he work, have found absolute confirmation as our knowledge of immunity, toxins and antitoxins progresses. When we remember that Pasteur made the first application of this method of treatment to human medicine October 26, 1885, six years before the time we began to understand the production and use of antitoxins, it becomes one of the most remarkable achievements of medicine.

Pasteur, then, introduced the principal of accustoming an animal first to very small doses of poison or attenuated virus, then to stronger and stronger ones, until immunity was established, while his treatment of the disease consisted simply in the production of this immunity during the incubation period of the affection, so that the subsequent course of the disease was prevented. It was upon this same principle that Behring subsequently began his work upon diphtheria immunization. It was in 1890 that Behring announced his discovery that the blood of animals rendered immune against diphtheria by the inoculation, first of attenuated and then of virulent organisms, contained a neutralizing substance capable of annulling the effects of the bacilli or their toxin when simultaneously or subsequently inoculated into susceptible animals. This substance, held in solution in the bloodserum of immunized animals, is the diphtheria antitoxin.

The antitoxin is manufactured at present on a commercial scale by immunizing horses against increasing amounts of diphtheria toxin, until the proper degree of immunity has been attained, then withdrawing the antitoxic blood. The bloodserum of this blood withdrawn from the immune horse is the antitoxin of commerce, which introduced beneath the skin of a person who has the disease will result in a cure or when introduced into a person who has been exposed to the infection will result in the prevention of the disease by the production of immunity to it.

That the use of antitoxin in diphtheria has proved itself of great value in reducing the mortality from diphtheria and in diminishing the severity of the disease is now admitted by all intelligent observers. As proof of its value it is but necessary to study the death rate from this disease in the cities and hospitals of the civilized world, and to note its reduction since the introduction of diphtheria antitoxin.

Before antitoxin was used the death rate from diphtheria varied from thirty to fifty per cent. In 11,000 cases treated in one hospital from 1888 to 1894 inclusive, the death rate was thirty per cent. From 1895 to 1898 in the same hospital, 20,000 patients were treated with antitoxin and the death rate fell to eighteen per cent. In the Boston City Hospital before the use of antitoxin the death rate was forty-six per cent.; after the use of antitoxin twelve per cent. We might multiply statistics but it would not add to our case. The brilliant results from the use of antitoxin are today generally admitted, and it is with perfect justice that diphtheria antitoxin has been hailed as one of the most valuable additions to medicine.

Just as the wine industry and the silk industry of France were saved from destruction by the brilliant achievements of Pasteur, so in our own country and in our own time an important industry has been saved by the work of the biologists connected with our national Department of Agriculture.

The popular conception of insects in general is undoubtedly that they are injurious. Many writers, it is true, have pointed out the benefits derived from insects, but usually we think of their damage to crops and of their annoyance to man and animals, and this aspect of the subject is apt to preponderate in our minds. In truth the economic bearings of insect enemies of insects are very great, and perhaps, this is, all things considered, the most important of the beneficial functions of insects as a class.

In the eternal warfare of organism upon organism, in the perpetual strife of species, one preying upon another and that upon a third, the complications of relations which determine the abundance of one species and the scarcity of another, are nowhere more marked than among the insects. There is not a species of insect that has not its natural enemies in the guise of other insects, there is not one of those other insects which has not its own insect foes. Outbreaks of injurious insects are frequently stopped as if by magic by the work of insect enemies. The cotton worm in Florida, the shade-tree destroyer in Washington, the Hessian fly in the wheat growing states are all held in check by their insect enemies. It is the work of the biologist to study these relations of parasite and injurious insect, to learn to control and regulate them.

In 1873 an American predatory mite was introduced into France to control a grapevine pest. In 1874 plant lice parasites were sent from England to New Zealand. In 1883 the European parasite of the imported cabbage worm was introduced into this country. In 1891 European parasites were introduced into this country to control the Hessian fly.

But all previous experiments in this line were dwarfed into insignificance by the astounding success of the importation of a ladybird beetle from Australia into California. The orange industry of the southwest was threatened with total destruction. A scale insect was spreading rapidly from orchard to orchard, leaving ruin in its wake. Something must be done and done quickly. The national Division of Entomology of the Department of Agriculture, in conjunction with the State Board of Horticulture of California, set the best entomologists of the land at work on the problem. It was soon learned that this destructive scale had been introduced into California from Australia. In

Australia it was doing no damage and why? It was held in check by its natural enemies. Why not introduce those enemies into California? The experiment was tried. After several years the ladybird enemies of the scale, established themselves in California. The story of the care and nurture of those first broods of ladybirds is interesting. Little by little they gained in numbers; orchard after orchard was invaded, and everywhere they went the scale insects were devoured and destroyed. The orange industry of California is now safe, the ladybirds are everywhere, thanks to the experiments and tireless work of the biologists of the Department of Agriculture.

More recently still the same insect has been introduced into South Africa and into Egypt. Another ladybird has been introduced into the olive groves of this country and has rid them of the destructive black scale. Into the Sandwich Islands has been introduced a beetle which has exterminated the plant lice of the sugar cane, and another which has saved the coffee plants from total destruction. In return for our own success with the ladybirds on the California orange trees, we have sent the same insect to Portugal where it is continuing its good work by rescuing the orchards of that country from another destructive scale.

It is in line with this work that we read of the Guatemalan ants imported into our Southern States to protect our cotton fields from the cotton boll weevil. It is too early to speak of the success of this experiment, but it is along these biological lines that we must expect success, not only with the cotton boll weevil, but with the gypsy moth, the brown-tail moth, the San Jose scale and the other pests which threaten the agricultural interests of different parts of the country from time to time. And it may be also along these lines that we should look for deliverance from those arch enemies of ours, the house-fly and the mosquito.

It was in the same state of California that it was hoped that the Smyrna fig might be grown successfully. Climatic conditions appeared to be perfect. The trees were planted and grew apace; they budded and fruit set, but soon fell off. Again and again the experiment was tried but fruit could not be matured. The biologist was called upon. A study of the fig in its native land soon showed what was the matter. A little fly was the bearer of pollen from the stamens of one fig flower to the pistils of another. Without this fly no figs could mature. It was then plain that this fig-fertilizing insect must be introduced into California. The Division of Entomology again brought honor upon itself by successfully establishing the insect in California. In 1900 eleven tons of Smyrna figs were raised in California, were dried and placed on the market, of a quality superior to the imported fruit. The insects were successfully carried through the winter of 1900-1901 and in the autumn of 1901 the crop of figs was so abundant that it was difficult to estimate. This number has been increased materially in the last few years.

The botanists too have played their part in biological research. We have grown accustomed to the idea of being vaccinated, but to cure sterile ground and make it bring forth fruit in abundance by inoculation is something strange and revolutionary. This the botanists have done. Before explaining the discovery and manner of this extraordinary process of agricultural science it might be well to review a few well known facts in the life of plants.

One of the most important elements of the food of a plant is nitrogen, which it absorbs from the soil mainly through its roots; the nitrogen of the air, though so abundant, is not at all available as plant food; plants have not the power of using it. So successive crops soon drain the soil of its plant food and in the process of time make the richest land poor and worthless. We are taking nitrogen from the soil so much faster than we can put it back; so much is daily wasted, washed into the ocean and lost forever, so much is dissipated into the air, our sources of nitrogen are so limited, the phosphate deposits and the guano islands are so nearly exhausted, the amount wrested from the atmosphere by the electricity generated at Niagara Falls is so small, that some persons predicted a "nitrogen famine" at no distant day and have luridly described the horror that will fall upon us when the soil becomes so poverty stricken that our crops of wheat and corn and rice will fail to feed the nations.

But the botanist has captured a tiny germ, invisible to the naked eye, which can take this valuable element from the boundless store of nitrogen in the atmosphere and put it into the soil in a form available for plant food.

If one digs up a healthy bean or clover plant and examines the roots, he will see a number of nodules or tubercles on them. On dissecting one of these and examining it under the microscope it is found packed with bacteria. These bacteria are incessantly absorbing free nitrogen from the air and converting it into forms suitable for plant food. This is why the farmer sows his land to clover and plows in the crop. He has done this from time immemorial, not because he knew that the bacteria were there, but because he knew that it increased the fertility of his soil.

A celebrated German biologist, Professor Nobbe, realized that if he could put into barren ground some of these nitrogen fixing organisms he could make plants grow in the most hopeless soils. But he was not able to make the bacteria work the miracles promised. The vast majority of his experiments were failures. At this point the inventive genius of an American, Dr. George T. Moore of the Laboratory of Plant Physiology of the Department of Agriculture, came to the rescue. He was able to produce by cultivation a type of organism with five or ten times the power of fixing nitrogen that the original germs possessed. He further found that it was possible to allow small pieces of absorbant cotton to soak up millions of these organisms and then by allowing these cultures to become dry, the bacteria could be sent to any part of the world and yet arrive in perfect condition.

Naturally Dr. Moore patented his discovery, but then he did a very unusual thing. He deeded the patent to the Department of Agriculture, in trust for the American people. A proper germ and a simple method of distributing it having been found, it was announced that the Department of Agriculture was prepared to send applicants, free of charge, enough inoculating material for several acres. Enough germs were sent in each package to inoculate seeds for from one to four acres. The package can be carried in your pocket and yet does more work than several cartloads of fertilizer. It costs the government less than four cents a cake, or less than one cent per acre, and saves the farmer thirty or forty dollars which he would otherwise have to spend for fertilizer.

The results have been surprising. To give but one illustration, a patch of crimson clover under similar conditions of cultivation, uninoculated yields 372 pounds, inoculated yields 6,292 pounds, an increase of nearly twenty times.

It does not require a trained bacteriologist to apply the cultures. The results obtained by any intelligent farmer are as wonderful as these. There is not a section of the United States that will not profit by Dr. Moore's discovery. Nearly every state has its worn out farming land, bringing despair to the economist who laments our careless handling of the fields and who wonders how the country will support the hundreds of millions of people soon to be ours. These bacteria mean intensive cultivation with a vengeance, and should give him hope. It is impossible as yet to calculate by how much this discovery will enhance the yield of our crops and of the world's crops, but the results already achieved prove that in time the gain will be enormous.

I know of no better illustration of the work of the biologist and no better demonstration of the fact that fragments of truth wrested from the unknown by careful research may form the connecting links of a great discovery, than the problem of the cause and spread of malaria and the relation thereto of the mosquito.

Four classes of men have taken a part in this work. The first step was the study of malaria itself from the standpoint of the physician. This resulted in the year 1880 in the discovery of the protozoan parasite of malaria in the blood of those suffering from the disease. No sooner was this discovery made than the medical biologist and zoologist brought to bear upon the question their knowledge of blood parasites in general, their distribution, their life history. Little observations made here and there in years past, studies of parasites of other animals than man, all added their quota to the matter in hand.

As the result of this putting together of the evidence in hand came the discovery that the malarial parasite undergoes part of its life cycle in the mosquito; this discovery necessitated the study of the distribution, habits and varieties of the mosquito by zoologists and entomologists. And finally came the investigation of the best way to get rid of the mosquito, which is the work of the entomologist and the sanitary engineer.

It was Ross, an Englishman, working in India, studying the development of the blood parasites of birds who first definitely established the fact that part of the life cycle of these parasites must be passed in the body of the mosquito, and that it is only by the bite of a mosquito that the disease may be transferred from one bird to another. What was true of the malarial parasite of birds was soon found to be true of malaria in human beings. The life cycle of the malarial parasite of man was found to be passed in part in the body of the mosquito. The biologist was called upon for his knowledge of the habits, life history, classification of mosquitoes. But very little had been done with the mosquito previously, but with renewed interest the entomologist was soon able to tell us all the facts necessary to enable us to begin a warfare upon this previously unwelcome pest, now known to be the bearer of disease.

As the result of this work, in many parts of the world mosquitoes, to a large extent, have been exterminated. The Roman Campania, a place once most deadly, has now become habitable; malaria, so long a foe to the spread of European empire, has been conquered, and any locality that still endures the presence of the disease laden mosquito is certainly far behind the times. Providence, I am sure, could be free from mosquitoes and free from malaria to a large extent, were the people once awake to the fact that modern biologists have made it possible to exterminate both insect and disease.

But the bearing of this mosquito study has gone farther than this. In 1881 Dr. Carlos Finlay of Havana noticed a correspondence between the abundance of mosquitoes and a period of increase of yellow fever in the autumn, while during the summer yellow fever had been scarce and mosquitoes also scarce. This suggested to him the idea that mosquitoes are responsible for the transfer of this disease also. He conducted certain experiments and proclaimed that he had produced a case of the disease by the bite of a mosquito. Dr. Finlay's theory was received with interest by physicians and investigators, but with very pronounced general incredulity. His published papers did not excite sufficient interest to induce other investigators to undertake any experimental work in the same direction. When, however, the beautiful results of the malarial investigators had become generally accepted, then it was that a few people began to think of Dr. Finlay and his theory of yellow fever.

The occupation of the Island of Cuba by American troops attracted the attention of Dr. Sternberg, then Surgeon-General of the Army, more strongly than ever before, to the subject of yellow fever; and he established a commission of army surgeons composed of Drs. Reed, Carroll, Lazear, and Agramonte and instructed them to investigate the disease from the mosquito standpoint.

In the course of the investigations both Drs. Carroll and Lazear were attacked by the disease. Dr. Carroll recovered, but Dr. Lazear died, another of the long list of martyrs who have suffered in the cause of scientific research.

The conclusion of this commission was that the only way that yellow fever could be contracted was through the bite of a certain variety of mosquito that previously had bitten a yellow fever patient, and that the spread of yellow fever could be controlled most effectually by measures directed toward the destruction of mosquitoes, and the protection of the sick from the bites of these insects.

Major General Wood, himself a physician, was quick to make use of the conclusions of this commission. In response to his attempts to bring about a hygienic revolution in the island, the Cubans were not slow to proclaim that yellow jack would prove too much for the Americans and their sanitation. But mosquito bars were ordered erected about every case of the disease, no new cases were allowed to enter the city, mosquito breeding places were drained or treated with oil.

Before 1900 there had been reported in the city of Havana 35,952 deaths from yellow fever. Within ninety days after these regulations were put into force, Havana was free from yellow fever. The disease has not caused any alarm in Havana since, and for practically three years there have been no cases of the disease reported, this too, notwithstanding the fact that when yellow fever is imported into the city no special precautions are taken to prevent contact with patients, and the only sanitary regulation is the absolute seclusion of the yellow fever sufferer from mosquitoes.

These investigations of Reed and his associates on yellow fever have resulted in the saving of more lives annually than were lost in the entire Spanish war; and what is quite as noteworthy, the annual saving to the tropical regions of our Southern States, because of the abolition of quarantine, of a sum greater than the whole cost of our conflict with Spain.

Our government has on hand at the present time an undertaking which will equal if not surpass the work done in Cuba. The Panama Canal is to be dug in a tropical country, and not the least of the difficulties to be overcome is the prevalence of disease among the workmen. Malaria and yellow fever are two special sources of danger that must be avoided, if every foot of the canal is not to cost a human life. The whole effort of the large sanitary corps of the canal is to be centralized in the single purpose of preventing mosquitoes from spreading these diseases from the sick to the well.

All this looks simple enough now. The very favorable outlook, however, is due entirely to the work of those biologists, who, in a quiet, unassuming way, studied blood parasites and mosquitoes, without an idea of the wide fruitfulness of researches, the immediate purposes of which were narrowly defined. And so we are pleased to note that practical men are coming daily to recognize the value of researches which do not appear at first to minister directly to their purposes. On all sides investigations are in progress, inspired solely, in the beginning, by a search for truth, but ultimately leading to the material advance of applied science.

THE METAMORPHOSES OF THE HERMIT CRAB.

BY M. T. THOMPSON.

Proceedings of the Boston Society of Natural History, Vol. 31, No. 4,
pp. 147-209, pl. 4-10, September, 1903.

A Thesis submitted to the Faculty of Brown University
for the degree of Doctor of Philosophy.

Proceedings of the Boston Society of Natural History.
Vol. 31, No. 4,
p. 147–209, pl. 4–10.

THE METAMORPHOSES OF THE HERMIT CRAB.

[Thesis Submitted to the Faculty of Brown University for the Degree of Doctor of Philosophy.]

By Millett T. Thompson.

BOSTON:
PRINTED FOR THE SOCIETY.
September, 1903.

No. 4.—THE METAMORPHOSES OF THE HERMIT CRAB.[1]

BY MILLETT T. THOMPSON.

Introduction.

The group of the Pagurids or hermit crabs has always attracted the attention of carcinologists, not only because of its extent both in species and in individuals, but also because of the asymmetry which involves nearly every genus and the habit of protecting the soft abdomen within hollow objects, typically the shells of Gastropod Mollusca. Whether the asymmetry — which is of dextral type except in the genus Paguropsis — owes its origin to this use of shells for residences, since nearly all marine Gastropod shells are dextral in coil, cannot be determined with certainty until the phylogeny and relationships of the various genera are better understood. But, nevertheless, it is unquestionable that the modifications found are in a very great degree correlated with residence in dextrally spiral shells. This alone makes the ontogeny of the group an extremely interesting and important subject for study.

Knowledge of Pagurid ontogeny was in its beginnings practically contemporaneous with the discovery of the metamorphosis among the higher Crustacea. During the discussion which followed Thompson's assertion ('28) that the supposed genus "Zoea" was a larva, Vigors ('30) in a review of Rathke's study of the development of *Astacus fluviatilis*, which has no metamorphosis, appeared skeptical with regard to Thompson's conclusions. This drew a reply from the latter author (Thompson, '30–'31), communicating a list of fifteen Decapods in which he had observed the young to be unlike the parent and "Pagurus" was included in this list.

The further statement by Thompson five years later, in 1835, that both "Zoe" and "Megalope" were larvae and his reiteration that, among "Macrura," *Astacus marinus*, Pagurus, and other forms underwent a metamorphosis, greatly stimulated research on crustacean development, and in the year 1840, two papers on Pagurid development appeared. One of these (Rathke, '**40**) deserves to stand as the first real contribution to our knowledge of the metamorphosis in this group. For Thompson did not describe his larva and Philippi's paper of the same year merely figured in a rough way the first zoea. Rathke's paper, however, briefly described three zoeal and a Macruran-like older stage of the European "*Pagurus bernhardus.*" Two years later (Rathke, '**42**) it was republished in more complete form with excellent figures of the zoeae and of the maxillipeds, tail fan, and pleopods of the "older larva."

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[1] From the Anatomical laboratory, Brown university, and the laboratory of the U. S. fish commission, Woods Hole, Mass.

Then twenty years passed without any important addition along this line of carcinological research until the publication of Müller's "Für Darwin," in 1864. This article described a "Pagurus" zoea, called attention to the absence of any gradation in the successive zoeal stages towards the Macruran-like stage, and, although it is uncertain whether Müller actually saw this later stage, it is described and compared to a small shrimp which had received from Milne-Edwards the name Glaucothoe. "*Glaucothoe peronii* may be such a young, still symmetrical Pagurus." The recapitulatory nature of the stage is also asserted. "The abdomen is truly in the adult a clumsy [ungeschlachter] sac, filled with liver and sexual organs, but it is yet fairly powerful [kräftig] in the glaucothoe-stage and it was also still stronger when this stage was the permanent form of the animal."

A few years after Müller's article appeared, Spence Bate ('**68**) published an account of two zoeae and a glaucothoe which he collected at the surface and correctly assigned to "Pagurus." Of the latter stage he says: "In this they probably continue until they find a suitable molluscous shell. . . . I imagine that they may cast their exuvium and grow during the whole time that they are deficient of such a shell because I have taken specimens, occupants of shells, that were still smaller than the ones described and yet further advanced toward maturity. It would be curious to see if, were they deprived entirely of a shell as a habitat they would continue to grow and retain the normal [*i. e.*, symmetrical] form of the pleon generally."

This query as to the effect of depriving the young of a shell was in part answered by Agassiz ('**75**) nearly ten years later. He showed that the larva might attain asymmetry and a soft abdomen before a shell was entered and considered that the desire for a shell arose from the anatomical changes. The extreme brevity of his record, however, has been a cause of much confusion. It was not clearly shown whether this metamorphosis without a shell was the typical developmental sequence or only a frequent happening. Moreover, the amount of the asymmetry attained at this time was not certainly defined. Hence the record has been interpreted to mean that the change from the glaucothoe to the adult form was gradual and covered several ecdyses (Bouvier, '91). But I am convinced that Agassiz intended to indicate the change as occurring with a single ecdysis and the figures published by Faxon ('82) support this contention. These show four zoeae, and in less detail a fifth stage which is identified with the genera Glaucothoe and Prophylax. Immediately following the fifth stage comes a stage entitled "age when it takes up its abode in a molluscan shell," and this figure depicts a crab with almost adult asymmetry, but bearing minute rudiments of pleopods on the right side of the abdomen.

The main outlines of Pagurid ontogeny had now become clear. The young passed through several zoea stages and then moulted to a glaucothoe phase analogous to the megalops of the true crabs, and as with them the mysis phase was suppressed (Claus, '76) into the last of the zoea stages, the metazoea (Claus, '85). The anatomy of the glaucothoe, however, remained almost unknown and the details of the metamorphosis to the adult form obscure. For as already noted, considerable uncertainty existed as to whether the larva passed to the adolescent phase directly or gradually. Since Agassiz's note, no work has given us much additional data regarding this most important point in the whole development. For the recent articles by Sars ('89) and Czerniavsky ('84) do not deal with the postzoeal stage except to depict the external form, although they are valuable records for several genera and species of hermit crabs.

The present research, then, was undertaken in the hope that through a study of the anatomy from the time of hatching until the adult form was attained, and through examination of the *rôle* of the shell in the development, some knowledge of this almost untouched field might be obtained. The study was carried on during the three years prior to 1902 at Brown university and at the laboratory of the United States fish commission at Woods Hole. I wish to express my especial gratitude to Dr. H. C. Bumpus, now director of the American museum of natural history, for the assistance and guidance furnished me throughout the work at both institutions. I am also indebted to Dr. A. D. Mead, of Brown university, and to Dr. H. M. Smith, of the United States fish commission, for ample provision extended to me during my study.

THE ADULT CRAB.

Throughout the region about Woods Hole, the genus Eupagurus is practically supreme and is represented there in the shallower waters by four species, *viz.*: *longicarpus*, *annulipes*, *acadianus*, which is apparently only a variety of the European *bernhardus*, and *pollicaris*. Of these, *E. longicarpus* is the only one generally distributed along the shore. It is extremely abundant and extends from tide-water to a depth of about twenty-five fathoms and is associated over the lower limits of its range with *acadianus* which is a deeper-water species, and in the shallow waters with *pollicaris* and *annulipes*, but of these only *pollicaris* occurs along shore and then only in a few localities.

Because of its distribution and abundance, *E. longicarpus* was selected for the present research. But after much of the work had been completed, it was discovered that the larvae of *annulipes* could not be distinguished from those of the selected species, as their slightly smaller size furnished no adequate criterion for their separation. Whenever the *annulipes* larvae were unusually abundant, as was shown by the occurrence of their adolescent stages in the experiments, I could note no difference in the ontogeny from the periods when undoubted *longicarpus* predominated. In sections also, the smaller specimens of any stage, presumptive *annulipes*, were wholly like their larger companions. This is perfectly in accord with what we know of the adults themselves. *Eupagurus longicarpus* and *E. annulipes* differ only in specific details. *E. longicarpus* is the larger, has slender chelipeds and its coloration is diffuse. *E. annulipes*, on the other hand, has stout chelipeds and prominent belts of brown pigment on the anterior thoracic limbs. My research is therefore not invalidated, but rather enriched by the confusion. It becomes a life history of two, instead of one species.

The majority of the larvae handled belonged to *E. longicarpus* and it is fitting therefore that this species should be described rather than *E. annulipes*.. But the following account is applicable to the latter and to the other species of the genus also.

Like many other Decapods, *Eupagurus longicarpus* is crepuscular and during the day a majority of individuals remain buried in the sand or congregated in the shade. They are omnivorous and must glean very closely, as they pick up bits of gravel or detritus from the bottom and brush them over between the maxillipeds. They also toss sand — usually with the smaller cheliped only — to the mouth parts, brush it between them and let the grains fall again in a continuous stream. Probably it is in this manner that they obtain the diatoms and foraminifera, which are found in the stomach and intestine. But although the food is thus very largely diatomaceous, no sort of vegetable or animal matter is refused.

The asymmetry is dextral. The chelipeds and the two following pairs of limbs are larger on the right than on the left side. The abdomen is spirally twisted to the right and only the sixth segment, telson, and first segment are heavily calcified. The remainder and major portion is soft and bloated. The boundaries of the segments are lost, except dorsally where the posterior borders of the terga can be traced, and show that the fourth and fifth segments are elongate, while the second and third are shortened. The sternum of the first segment or peduncle is indistinguishably fused with the sternum of the last thoracic segment. The peduncle bears no appendages and the four succeeding segments have no pleopod on the right side. On the left side, the *female* has a large biramous pleopod on the second, third, and fourth segments and a smaller pleopod with a minute internal ramus on the fifth (pl. 6, fig. 24); while the *male* has a pleopod similar in shape to the most posterior of the female's series on the third, fourth, and fifth segments, but no pleopod on the second segment (pl. 6, fig. 25). The uropods are alike in the two sexes and the left is the larger (pl. 7, fig. 31_a).

The greater development of the female's pleopods is probably correlated with their use during the breeding season when the eggs are borne on the hairs of their borders. It must not be hastily assumed, however, that the pleopods of the male have no function. While watching adolescent crabs that were inhabiting straight glass shells I have noticed that their pleopods were at times waved in the water. This suggests a possible function for these appendages in both sexes to reinforce the current in the shell which is primarily induced by the branchial outflow and movements of the body. Bate ('50) records a similar movement of the appendages in female hermit crabs which were bearing eggs. The *rôle* played by the sensory hairs on these appendages must be nearly equivalent in both sexes.

A large columella prominence is present. Although it has been suggested that this organ aids the crab in maintaining a firm hold on its shell, I feel that this cannot be a complete explanation for the existence of the structure. If it serves this function at all, it must be mainly passively, in conforming the body more perfectly to the columella angle of the shell chamber. Moreover, it is not a very muscular organ even in the genera in which it is well developed and it is only imperfectly developed in a large number of hermit crabs. Our species of Eupagurus maintain their hold on the shell chiefly by the grip of the calcified telson and uropods on the columella, while the tuberculated areas on the posterior thoracic limbs and uropods may lend assistance. The chitin over the venter of the abdomen also is roughened with fine transverse lines. When an attempt is made to dislodge a crab by traction on its limbs, an additional resistance is often obtained by the elevation of the rostral region of the carapace against the roof of the chamber.

The breeding period of *E. longicarpus* is very long. Females with eggs attached to the pleopods can be obtained from May until mid-September. The zoeae begin to appear in the *auftrieb* in the latter part of June and are very abundant during July and August. The glaucothoe can be obtained as late as October. Our knowledge of the breeding period of the remaining species of Eupagurus is imperfect. *E. annulipes* breeds over practically the same period as *longicarpus*, ceasing a little earlier in the fall. Zoeae that agree closely with Sars' ('89) description of the zoeae of *E. bernhardus* occur scatteringly throughout the summer. Quite likely these are the larvae of *acadianus*. The glaucothoe, however, has eight instead of the ten telson bristles shown in Sars' plate. The young of *pollicaris* have not been identified and most of them are probably liberated in June, though females with eggs are taken during July.

General Account of the Metamorphosis.

The eggs and young of Eupagurus are very sensitive. Zoeae were hatched in confinement with great difficulty and as they invariably died in a few hours it was necessary to collect all material directly from the *auftrieb*. Even zoeae collected in this way could not endure more than one moult and although the later phases were much more resistant, they were nevertheless delicate as compared with the young of many other Decapods.

The most vital factors in rearing crab larvae seem to be cleanliness and an even, moderate, water temperature. The following method for rearing the young, although not original, is recorded here because it was by far the most satisfactory of those tried. In the end it was exclusively used. The young were kept in covered dishes of clean sea-water which was in sufficient volume to render unnecessary the use of algae for aeration. The water was changed daily, or in hot weather oftener, and to ensure a constant temperature the dishes were partly immersed in running water or suspended in large aquaria. Diatoms, collected with a fine net or scraped from submerged objects, were the most satisfactory food, but animal food was also given.

Woods Hole offers especial advantages for the study of crustacean development. Strong currents prevail in the neighboring waters, and one of these rushes past the wharves of the Fish Commission station during part of each tide and a large "tow net" may simply be suspended in this current and emptied at convenient intervals. Also, at those times when there is a paucity of animal life in the water, the numerous "slicks" caused by conflicting currents and back-sets, may prove an excellent resource, as the plankton is concentrated in these areas.

Zoea phase.— The zoeae of *longicarpus* and *annulipes* (pl. 4, fig. 1–4) have the characteristic Pagurid form: without carinae or spines; with long, straight rostrum; hind angles of carapace produced; swollen compound eyes, and third maxilliped rudimentary at time of hatching. The transparent body is pigmented with contractile scarlet and yellow chromatophores, and the eyes are black with yellow pigment diffused over the surface. No trace of a median eye can be found. The stomach and intestine are usually clean, as but little food is taken during the phase. The livers contain strongly refractive, yellowish globules. These larvae are phototactic, though to a less degree than Brachyuran zoeae, and in a glass vessel range themselves head downwards against the lighted side. They swim tail foremost, and rather slowly and steadily. The surfaces of the body, however, are not definitely oriented, though either the dorsal or ventral are uppermost more frequently than the lateral. This lack of orientation seems to be correlated with the complete absence of otocysts in the three earlier stages and with the undeveloped condition of these organs in the fourth stage or metazoea. While swimming, the eyes, antennae, and uropods are invariably held in the positions shown in figure 4 (plate 4).

In captivity, the moults between the successive stages usually take place at night or in the early morning, and the larvae sink to the bottom of the aquarium and remain quiescent for a considerable period previous to the actual ecdysis. The manner of moulting under natural conditions could not be determined.

The length of the zoea phase and of its several stages is not known. The occurrence of the successive stages in the *auftrieb* indicates that the phase possibly extends through only two or three weeks. But little reliance, however, can be placed upon data of this class, and it is very probable that the period varies with the external conditions, as is the case with other crustacean larvae.

In our Eupagurids, the zoea phase comprises four stages, which may be separated as follows: —

First Zoea.

Third maxilliped rudimentary (pl. 4, fig. 1); sixth abdominal segment not distinct from telson; exopods of maxillipeds with four feathered setae; thoracic limbs as a simple mass of undifferentiated tissue. Length, 1.9–2.7 mm.[1]

Second Zoea.

Exopod of third maxilliped functional as swimming-foot; endopod barely indicated (pl. 6, fig. 14_2); exopods of maxillipeds with six setae; *anlagen* of uropods as a band of tissue along each side of the telson (pl. 7, fig. 31_2); thoracic limbs distinct, rudimentary, fourth pair excessively long, fifth pair short and concealed beneath others (these proportions are found also in the two following stages). Length, 2.7–3 mm.[1]

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[1] Including as they do the larvae of two species, all the measurements have an unduly extended range.

Third Zoea.

Uropods present, and sixth abdominal segment distinct; exopods with seven setae; rudiments of the gills on second, third, and fourth limbs. Length, 3.5–4 mm.[1]

Fourth Zoea, Metazoea.

Limb rudiments very large, dactylus of cheliped distinct; rudimentary pleopods present; gill rudiments for chelipeds present; right cheliped obviously larger than left — this condition of the chelipeds may possibly date from the third zoea; maxillipedal exopods with eight setae; uropods symmetrical; mandibles without palpus rudiment. Length, *circa* 4 mm.[1]

Faxon (**'82**) described four zoea stages identical with these four, and it is likely that he studied larvae belonging either to *Eupagurus longicarpus* or to *annulipes*. Other investigators have not recorded so many stages. None of them describes the second zoea, unless one of the figures, no. 52, in Claus's "Zur Kenntniss der Kreislaufsorgane" (**'84**), corresponds to it. But it should not be assumed that this stage is typically absent from the zoea phase of the various Pagurids, for it closely resembles the first stage and might readily be overlooked. My third zoea corresponds to Rathke's (**'40**, **'42**) "young of one and three-fourths lines" and to Claus's (**'61**) "späteres Stadium." The other writers make no mention of a similar stage. The metazoea has been repeatedly noted. Rathke (**'40**, **'42**) termed it, "young over two lines long"; Bate (**'68**), "what we take to be the second stage"; Claus, "Mysisstadium" (**'76**), "Metazoea" (**'85**); and Sars (**'89**), "last larval stage," "last stage before moult to adolescent stage."

Postzoeal phase.— The postzoeal phase includes only one stage, the fifth or glaucothoe (pl. 4, fig. 5). The larva is now 2.8–3.3 mm. long and is Macruran in form with symmetrical abdomen and with pleopods on the second to the fifth segment. The uropods are asymmetrical as in the adult, and the thoracic appendages are on the whole adult in type and proportions. A few tubercles on the posterior thoracic limbs and on the uropods (pl. 6, figs. 19, 20; pl. 7, fig. 31_5) represent the future tuberculated areas. The sternum of

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[1] Including as they do the larvae of two species, all the measurements have an unduly extended range.

the first abdominal segment, which is not very wide at any time, can no longer be detected in sections. The otocysts are functional and orientation is definite. Internally the livers, lateral caeca, green glands, and sexual glands are thoracic. The unpaired intestinal caecum is lacking and the muscles and blood vessels are similar to those of Macrurous Decapoda.

In color, the glaucothoe resemble zoeae except that the branches of the yellow chromatophores form a fine network over the limbs and carapace and produce a grayish effect. As before, the stomach and intestine are usually empty and transparent and the livers contain refractive globules.

Glaucothoe are found at the surface, either swimming or clinging to floating seaweed and seem to be more abundant at night. When swimming, the dorsal surface is uppermost, the abdomen is extended while the limbs are either extended or hang stiffly down. Though phototaxis is still present, at times individuals cease swimming and crawl about on the bottom of the aquarium. These examine the objects in their path, and if they find a shell or other hollow object, may enter it and abandon the free-swimming life. Quite frequently, however, after a brief exploration the larva will recommence swimming. More rarely, a glaucothoe that has already entered a house will abandon it. As the sixth stage approaches, the desire for a covering for the body becomes stronger with the alterations in structure until it is almost impossible to keep the larvae "naked." They use all available objects or ensconse themselves in crevices. A house may be taken at any time during the phase, but a short period of free-swimming life is typical.

Not infrequently a glaucothoe will remove bits of rubbish from a shell, but I was not able to confirm Agassiz's observations ('75) where his young tore out and ate dead snails and then used the shells. Glaucothoe, according to my observations, take but little food. If, however, his reference is to adolescent crabs, they are quite voracious and might readily eat a dead snail, though I have observed nothing of the sort. It is, however, scarcely necessary to assume or suggest a causal sequence between this act and the use of the shell for a dwelling.

The glaucothoe stage as a rule lasts only four or five days, but during this time a profound modification of structure takes place. The livers, sexual glands, and green glands pass into the abdomen, the circulatory system is modified, and the muscles and pleopods degenerate, so that before the moult to the sixth stage closes the period, the anatomy has become adult in plan. This metamorphosis is not dependent on the presence of a body covering, but completes itself perfectly in larvae which are prevented from obtaining a shell. The whole animal also becomes less transparent, the chelipeds become white, and brown bands appear on the posterior pereiopods, a coloration recalling that found in the adult *Eupagurus annulipes.*

The moult to the sixth stage is preceded by a brief period of quiescence, and in the few cases observed, the actual ecdysis was rapid. Either the integument of the thorax is sloughed first and that of the abdomen later, or the entire integument is sloughed in one piece. The latter method seems to be the rule for the zoeal ecdyses, while the former is more frequently the rule in the adult, and the abdominal exuvium is usually badly torn in the process.

The glaucothoe stage is the "noch ältern Jungern" of Rathke (**'40**, **'42**); the "Glaucothoestufe" of Müller (**'64**); the "third stage" of Bate (**'68**); "stage when it seeks a shell" of Agassiz (**'75**), and the "first postlarval," "first adolescent" of Sars (**'89**). It is not mentioned by other workers with the exception of Czerniavsky (**'84**).

Adolescent phase.—"Adolescent phase" is more a convenient term under which to discuss the development during the earlier postlarval life, before the adult anatomy is fully attained, than a definitely limited period. There is also little to be gained by an attempt to separate the numerous stages which may be included within it. The crab has the adult structural plan before the close of the glaucothoe period, but all the organs must still undergo development to realize fully the adult structure. The length of this process varies widely in different parts.

Sixth-stage larvae are of the same size as glaucothoe, and the specific adult form may be attained before much growth occurs. After about forty days have passed, the young reach a length of from five to eight millimeters. The sixth stage lasts from six to twelve days, but the later moults are irregular and crabs of the same age may be very unlike in size and development. The manner of life in all the adolescent stages is that of the adult, and food is taken abundantly almost immediately after the moult from the glaucothoe.

The sixth stage retains the *annulipes* color, handed to it by the glaucothoe (pl. 4, fig. 6). With the seventh stage, however, the *longicarpus* young become separable from *annulipes* larvae, as in them the brown bands are lost. The full adult colors and the complete specific form are not attained until about the twentieth day from the glaucothoe. The sixth stage also has a nine-jointed antennal flagellum and certain retrogressive alterations in the mouth parts, which are legacies from the glaucothoe (pl. 5, figs. 10, 12, 13). The elongation of the flagellum is very gradual, the nine-jointed condition often persisting till the eighth stage, while crabs forty days from the glaucothoe have only 17 or 27 joints.

The metamorphosis of the pleopods during this period is of special interest. At no time are there any traces of appendages on the peduncle, which is interesting when we remember that some Pagurids, as for example, Sympagurus and Paguristes, have pleopods on this segment in the adult. Typically, the sixth stage has no appendages on the right side of the abdomen, except the uropod, but on the left the pleopods are well developed on segment three to five and are of the type found in the glaucothoe, *i. e.*, they resemble those of the adult male. On the second segment the pleopod is reduced to a mere rudiment (pl. 4, fig. 6). About nineteen percent of reared sixth-stage larvae, however, retain rudiments of one or more of the right hand pleopods. The typical reduction may be expressed in a formula, by use of R for an appendage which is retained intact, Ru for a rudimentary appendage, and O to denote the loss of an appendage. The non-typical reductions are dealt with in another part of this paper.

	Glaucothoe.	Sixth Stage.	Adult Male.	Adult Female.
	Left : Right	Left : Right	Left : Right	Left : Right
Seg. 1	O : O	O : O	O : O	O : O
“ 2	R : R	Ru : O	O : O	R : O
“ 3	R : R	R : O	R : O	R : O
“ 4	R : R	R : O	R : O	R : O
“ 5	R : R	R : O	R : O	R : O
“ 6	+R : R—	+R : R—	+R : R—	+R : R—

At the moult to the seventh stage or, more rarely, at the next following moult, the retained rudiments are lost, except the one on the second segment in those crabs which will become females. But although sex can be recognized thus early, not less than a year and probably a still longer time must elapse before sexual maturity is attained. In the young females the rudiment on the second segment begins to develop into a perfect pleopod at about thirty days from the glaucothoe. The remaining pleopods, and in many instances, this one also, do not begin to alter to the female type until ten days later, and the development of this type requires several moults for its full completion.

The sixth stage was figured by Faxon ('82), and described by Agassiz ('75) as "stage when they need a shell." The later adolescent stages have not been recorded by anyone.

Special Account of the Metamorphosis.

The account of the development that has already been given has briefly described the plan of the anatomy in the larval stages and shown how this gives place to the adult type of structure in the glaucothoe phase. In the present chapter it is proposed to discuss in more detail the larval anatomy and the modifications by which the adult type is produced.

Technique.— In this work, microscopical examination of living larvae or of specimens cleared in cedar oil gave only dubious results and therefore it was necessary throughout to employ serial sections. No killing fluid was uniformly satisfactory because of the difficulties of penetration and because the same tissues in different stages do not react in the same way to a reagent. The best and most reliable solutions used were a saturated aqueous solution of picric acid, five percent picro-acetic, and the stronger solution of Flemming. The brown stain produced in the contents of the liver cells was a serious objection to the use of the otherwise excellent reagents of the vom Rath series, and the lime salts in the integument in all stages barred picrosulphuric. Perenyi's fluid and corrosive sublimate in its various forms, the latter admitted by incision, though they frequently gave excellent fixations, were uncertain in action.

To prevent injury to the delicate tissues after fixation, all the material was imbedded in paraffin from xylol as soon as possible. The integument in this connection offered no obstacle to penetration or dehydration. In sectioning, however, it was troublesome, forcing me to cut with a thickness of ten micra. Selective staining was found essential to good work, and iron-alum haematoxylin, counterstained with orange G or Bordeaux red proved especially valuable.

It was necessary to cut sections in both sagitto-longitudinal and transverse planes, for, although transverse sections are the more generally valuable, the others are essential for purposes of comparison and absolutely indispensable in studying the muscles of the abdomen. It was found advantageous, though not absolutely necessary, to have in addition to these last, a few corono-longitudinal sections of each stage.

External anatomy.— The development of the form of the body has already been in the main adequately treated in the preceding chapter, while the details of the development of the appendages will be better understood from the figures on plates 5, 6, and 7, than from any description. It remains, therefore, in this section, to speak of the telson and gills only.

The telson (pl. 7, fig. 31) in the first zoea bears on each side of the median marginal notch five feathered spines (1–5), a minute bristle (6), and the short, smooth spur of the angle (7). The formula is: 1, 2, 3, 4, 5, 6, 7. In the second stage, a pair of new spines (1′) are added within the older series. The tips of the uropods as they develop, are sheathed within the angle spur (7) and this is consequently lost when these appendages become free. The angle spur of the third zoea is a new structure (x). The third and fourth stages have the same telson formula: 1′, 1, 2, 3, 4, 5, 6, x, and in both, spine 4 is short and smooth. The eight setae on the border of the telson of the glaucothoe represent spines 1′, 1, 2, and 3 of the zoeal series. The median cleft appears with the sixth stage, and the adult form is attained with the seventh stage. The moderate length of spine 4 in the earlier, and its short spur-like form in the later zoea stages serve to differentiate the zoeae of *longicarpus* and *annulipes* from the zoea that I have assigned to *acadianus* and from the larva of the European *bernhardus* (Rathke, '**42**; Sars, '**89**), in both of which these spines are smooth and elongate throughout the zoea phase.

As already noted, the gills become functional with the glaucothoe stage. At this time they are present in the same number and arrangement as in the adult crab, *viz.*: —

Mxp_2	Mxp_3	I	II	III	IV	V		
0	0	0	0	0	1	0	1	Pleurobranch.
0	2	2	2	2	2	0	10	Arthrobranchs.
0	0	0	0	0	0	0	0	Podobranchs.

The larger posterior gills are provided with two short rows of ova lamellae, but the smaller anterior ones show at most only two or three plates each. The gills on the maxilliped and cheliped segments are simple and the maxillipedal pair are so minute as to be invisible in surface views. They cannot be detected, either, in some excellent sections. But since they are very plainly shown in all sections of mature glaucothoe and most of the sections in which they cannot be seen are of younger specimens, it may be presumed that they arise during the period. In this case it scarcely seems possible to refer their occasional absence to error in interpretation, although such outpushings of the body wall usually appear at and not between the ecdyses. Whenever present they are very distinctly shown in sections cut in any plane.

The sixth-stage larva has cheliped gills that are divided into two or three lamellae, but its maxillipedal gills are still simple. These latter reach a trilamellate condition at about the fortieth day from the glaucothoe phase. One crab eighty days from the glaucothoe phase showed an anterior maxillipedal gill with four, a posterior with twelve pairs of lamellae.

Internal anatomy.— A brief description of the stomach of the adult crab is a necessary preliminary to the description of the construction of this organ in the larvae, but it may be limited in scope to an account of the topography. No details of the ossicles are necessary, since these cannot be worked out in the serial sections of the developmental stages.

The stomach of the adult (pl. 9, fig. 57) has a cardiac portion of more than twice the length of the pyloric. The cardio-pyloric valve is crowned with blunt setae. Each lateral tooth consists of two rounded tubercles, a comb of transverse rugae, and a hairy terminal process. The oesophageal opening is guarded by a pair of upper and lower oesophageal plates. The pylorus is broadened laterally into a pair of shallow upper, and prominent lower pyloric pouches (pl. 9, fig. 57, 52, *upp*, *lpp*). The latter have their inner surfaces densely clothed with long setae, and above, the wall of the pylorus projects into the lumen in a prominent crest, the lateral-valve ridge (*lvr*), which terminates posteriorly as the lateral pyloro-intestinal valve. These valves are united with the dorso-lateral and the minute, median, dorsal valve for a space (pl. 9, fig. 58, *dv*, *dlv*, *lv*) so that these valves enter the intestine as a continuous curtain.

Throughout the zoea phase, the stomach (pl. 9, fig. 55) has a deep and narrow cardiac portion, which is not longer than the pylorus, and chitin is poorly developed except on the smooth cardiopyloric valve. There is no dorsal tooth; the lateral teeth are simple and project upward instead of horizontally (pl. 9, fig. 48, *lt*); the pyloro-intestinal valves are not united and are three in number, *viz.*: the paired laterals and dorsal. In the first zoea the pylorus is without pouches, but with the second stage an area on either side of the median pyloric valve becomes setose and in the fourth stage these areas are depressed to form the lateral pouches. The cardiopyloric and median pyloric valves are at first confluent, but become distinct with the second stage. A single oesophageal plate appears with the fourth stage.

The stomach of the glaucothoe (pl. 9, fig. 56) may be regarded as transitional in type, its more elongate form, horizontal lateral teeth (pl. 9, fig. 51), and well developed lateral-valve ridges recalling the stomach of the adult. There are indications of a dorsal tooth; the pyloro-intestinal valves are united; and a small dorso-lateral valve (*dlv*) is added on each side. In other respects the larval characters persist. No metamorphosis occurs during the period except a change in the mutual relations of the openings from the livers and lateral caeca.

The sixth stage has a stomach of adult type, but with the parts less specialized. The upper pyloric pouches are still wanting, and they are probably of late adolescent development.

In the intestine of the Decapoda, it is generally accepted that the limits of the chitinous lining are coincident with the limits of the post- and mid-guts. But until this is supported by a larger body of evidence, it is perhaps better to use the purely descriptive terms "chitinous" and "achitinous" for the post- and mid-guts respectively. In the adult Eupagurus the achitinous gut is relatively shorter than usual, as the chitinous gut only extends into the anterior part of the abdomen. An unpaired caecum — first described by Swammerdam in 1737 — starts from the right side of the achitinous gut in the rear of the thorax, turns back into the abdomen and lies there in a coil superficial to the livers (pl. 8, fig. 46, *ic*). The chitinous gut near the point of union with the anterior gut has the usual series of prominent folds, the methoria, and at its posterior end a rectum is differentiated. This occupies the sixth segment and the telson.

In the zoea and glaucothoe phases the achitinous gut is longer than in the adult and extends back to a point within the fifth segment of the abdomen, where its short, columnar cells give place to the larger, more vacuolated cells of the chitinous gut (pl. 9, fig. 54, *ch int*). Methoria are present at this point with the glaucothoe and during the latter part of this period the unpaired caecum arises as an outpushing of the dorsal wall of the achitinous gut just cephalad of these folds. As soon as this diverticulum appears, or occasionally a little earlier, the chitinous gut begins to encroach on the territory of the achitinous gut. Unfortunately, however, sections throw no light on the mechanism of the change, but a series of specimens merely shows the caecum, methoria, and chitinous lining lying farther and farther forward in the abdomen, until with the earlier adolescent stages the methoria reach their definitive position in the region of the second segment. No mitotic figures can be found and although histolysis occurs at this time throughout the length of the gut, it is not especially prominent.

The elongation of the achitinous gut which brings the proximal end of the caecum from the abdominal into its definitive thoracic position, must take place late in adolescent life. The diverticulum was still wholly abdominal in a reared specimen two months past the glaucothoe. But some small crabs that were collected at Wareham, Mass., in August, 1900, showed the caecum in its definitive relations. The age of these crabs was not known, but they were very small and their development was greatly advanced over that of larger, reared crabs known to be sixty days past the glaucothoe phase. So they were probably about a year old; perhaps two years old.

An elongate achitinous gut which is gradually replaced by the chitinous gut, is found in the young of other Decapods besides Eupagurus. For these, however, I have only fragmentary records. An examination of an immature Virbius, 4 mm. long, and of a Crangon with a length of 6 mm., shows no methoria, but the achitinous gut extends back as far as the fifth abdominal segment. A similar condition is found in Homarus even in the first adolescent — fourth — stage, at which time the animal is adult in form and has a caecum developed from the anterior gut. Among Thalassinids, the older zoeae of *Gebia affinis* and *Callianassa stimpsoni* have the union of the two regions of the gut in the fifth abdominal segment, and in all the preadolescent stages of Naushonia (M. T. Thompson, :03) this relation is maintained with the addition of methoria. The metazoea of *Hippa talpoidea* has the methoria in the anterior part of segment five of the abdomen, but in the first adolescent these have moved to the second segment. Among the Brachyura, the late zoea of Pinnotheres displays the anterior limit of chitin at the fifth abdominal segment and the megalops has methoria at this point. There is no caecum at this stage. On the other hand, a very young zoea referable either to Cancer or to Carcinus showed methoria and a well developed caecum in the second abdominal segment. The megalops of *Callinectes hastatus* likewise has these parts in this anterior segment and in this crab they have moved to the first segment in the first adolescent stage. *Cancer irroratus* has the caecum and methoria in the rear of the thorax in both metazoea and megalops.

The livers or enteric glands, which open from the lateral pyloric pouches are very voluminous in the adult crab. Each consists of an axial tube from which arise slender lateral diverticula. The latter are long and numerous along the abdominal portion of the axis, but short and scanty along the thoracic. Both axis and tubules have a wall of one layer of cells with abundant cytoplasm (pl. 9, fig. 53) vacuolated and laden with secretions. At intervals, single cells or groups of cells, either granular, or more usually vacuolated, project into the lumen and partly occlude it. Proper fixation of these tissues is difficult and they do not stain readily.

Immediately caudad from the openings of the livers into the pylorus, a pair of lateral caeca arise. These lie one on either side of the stomach in an irregular coil. The cells of their walls are distinguishable from those of the livers by the absence of secretions and vacuoles, and by the ease with which they may be fixed and stained.

During the zoea phase, the livers are cephalic in position (pl. 8, fig. 34–37). Each communicates with the pylorus by an extensive opening and presents four diverticula: anterior, lateral, dorsal, and posterior lobes. The cells of these glands are vacuolated and distended by deposits of a highly refractive yellow substance that stains black with osmic acid. As in the adult, cells or groups of cells may partly occlude the lumen, and during the fourth zoea and glaucothoe stages this phenomenon reaches its climax (pl. 9, fig. 58). The lateral caeca at this time are rounded glands which resemble the livers in histology and in reactions to reagents. Posteriorly they become approximated and enter the main canal dorso-laterally at the origin of the intestine, ten or even twenty micra cephalad from the openings of the livers (pl. 9, figs. 48, 49, 50).

The livers and lateral caeca of the glaucothoe have at first the same relations as in the zoeae except that they extend within the newly developed thorax (pl. 8, fig. 38). The lateral caeca are insignificant in size throughout the phase, and undergo no metamorphosis beyond a separation of their proximal ends, so that they enter the intestine at the sides of the stomach, as in the adult crab. The livers, on the other hand, pass through a complicated metamorphosis.

A description of a selected series of glaucothoe of different ages will better indicate the order of these modifications as well as the relations that they bear to the changes in position or structure in the other organs of the body, than a detailed account. The alterations are of course, subject to slight individual variations.

1. A young glaucothoe just moulted from the zoea (pl. 8, fig. 38). Only the posterior lobes of the livers have increased proportionally to the increased size of the stomach.

2. An older specimen, never in a shell (pl. 8, fig. 39). The anterior and dorsal lobes of the livers are further reduced; the lateral lobes have almost disappeared, though the one on the right still retains a minute lumen; the posterior lobes extend to the last segment of the thorax and the apex of the right gland lies beneath the intestine toward the left side of the body. The openings of the lateral caeca into the intestine are now caudad instead of cephalad from the openings of the livers. The green glands have begun to grow back to form the median nephrosac and a new artery is developing in the abdomen.

3. A still older specimen. The lateral liver lobes have disappeared; the dorsal become mere prominences, and the posterior barely enter the abdomen.

4. Next specimen. The posterior lobes are now abdominal, lying to the left of the intestine, which is displaced to the right and dorsally. The canals from the green glands reach the region of the pericardium but are not yet united. The sexual cells are abdominal. A rudimentary intestinal caecum is present.

5. With livers completely shifted (pl. 8, fig. 40). The dorsal lobes have disappeared, and the anterior are greatly reduced. The chitinous gut is rapidly elongating, advancing the caecum towards the anterior part of the abdomen. The green glands have formed the nephrosac. The muscles are beginning to degenerate.

6. Fourth day in the shell. The anterior lobes of the liver are gone. The cells of the lateral caeca have taken on the adult histology and reactions; the nephrosac is abdominal; the muscles and pleopods and all the other organs are of adult type, in readiness for the moult to the adolescent phase.

Sixth-stage crabs retain the simple cylindrical livers (pl. 8, fig. 42) for only a few days; then diverticula begin to appear along the borders, first of the right, then of the left gland (pl. 8, fig. 43). The development of these diverticula seems to follow a fairly definite plan (pl. 8, fig. 44). Owing to the way in which those from the right gland pass under the intestine, the adult condition is ultimately produced and the earlier displacement is obscured to casual inspection. The livers seem to lie each on its own side of the intestine. A shift of the latter back again toward the mid-line of the body, which becomes possible from the eighth stage on because of the gradual proportional increase of the diameter of the abdomen, also aids in confusing the earlier relations. But by careful dissection, the displacement can be traced in the adult. The main axis of the right gland will be found to lie beneath or slightly to the left of the gut for a considerable part of its course in the abdomen. As young crabs show this better than older ones, two views of the abdominal contents of the crabs collected at Wareham are appended (pl. 8, figs. 45, 46).

The green glands of *Eupagurus longicarpus* have the same general arrangement as the glands of *E. bernhardus* described by Marchal ('92), except that the nephrosac is short and broad and the canals which unite this structure to the cephalic portion of the gland are without accessory diverticula. The cells of the canals and nephrosac have a characteristic histological appearance (pl. 9, figs. 52, 53). The cytoplasm is scanty and stains faintly; the nuclei are small, spherical, and prominent.

The green glands cannot be found in sections of the first or second zoea stages. Some third zoeae show them, but they are without a lumen and almost wholly confined within the base of the antennae. These glands are, however, constantly present in fourth zoeae and each has the form of a bent tube, 0.2 mm. long, either simple or with two short, ventrally projecting diverticula at the proximal end.

The glands of the glaucothoe are relatively longer than those of the zoeae and extend out of the antenna vertically into the cephalothoracic cavity (pl. 9, fig. 51, *gg*). Their shape may be compared to a letter "L"; the orifice being situated at the angle, and the proximal diverticula forming the shorter arm. About the time when the livers swing to the left as they pass toward the abdomen, the tip of the vertical limb of each green gland begins to grow back as a canal which lies closely appressed against the lateral wall of the cephalothorax until it reaches the region of the pericardium. Here the canals swing toward the midline of the body, meet one another beneath the pericardial septum, and fuse to form a nephrosac, 0.1–0.2 mm. long (pl. 8, fig. 40), which for a considerable period may retain an imperfect median partition as a remnant of its double origin. Toward the end of the glaucothoe phase the nephrosac passes to its definitive position in the abdomen (pl. 8, fig. 42) and during the adolescent period attains to the larger proportions relative to the surrounding structures which it has in the adult crab.

The cells of the basal portion of these glands in all stages, and of the whole gland in the zoeae and glaucothoe, have granular, homogeneous cytoplasm, small, reticulate nuclei, and indistinct cell boundaries. But the canals and nephrosac from their first appearance show the adult histology. Though the bulk of the gland is not sufficient for the production of canals and nephrosac without a multiplication of cells, no mitotic figures could be found, which recalls the condition of the liver cells during the changes in those organs, where the nuclei remained reticulate. But there the original bulk is ample to form the livers of the sixth stage through a remodeling. In both series of specimens the tissues seemed perfectly well preserved.

A shell gland is present throughout the zoea phase. It is lost with the moult to the glaucothoe. It opens on the ventral face of the second maxilla and in form is a bent tube whose distal end extends toward the cephalothoracic cavity. These glands must be very important for the zoea before the development of the green glands with the fourth stage, and, despite the fact that the green glands have a lumen and are therefore presumably functional, in the latter period the shell glands are relatively longer than in the three earlier stages.

Our knowledge of the development of the sexual system is very meager. A sixth-stage larva or a mature glaucothoe shows in the abdomen near the tip of the nephrosac two fusiform clusters of five or six cells (pl. 8, fig. 42, *g*; pl. 10, fig. 63). These are readily identifiable as the sexual glands. Younger zoeae and glaucothoe have similar cells lying beneath the pericardial septum in the thorax (pl. 10, fig. 62). This position recalls the grouping of the sexual cells in the zoeae of Mysis, (Nusbaum, **'87**), Palaemonetes (Allen, **'93**) and Gebia (Butschinsky, **'94**) the only Decapod larvae for which they have been described. I was not able to find sexual cells in any earlier stage than the fourth zoea, perhaps because of the thickness of my sections. These cells pass to the abdomen at the time when the livers shift, but unfortunately no sections showed them in transition. There must, however, be an increase in their number at this time. The pericardial group contains less than half a dozen cells, while the abdominal clusters have five or more cells apiece.

The time for the appearance of sexual ducts and orifices is unknown. When adolescent larvae reach an age of about forty days from the glaucothoe, they show what are apparently sexual orifices. But if these specimens are sectioned, no openings can be found nor anything that can be interpreted as even the *anlage* of a duct. These "pseudo-orifices" must be merely shallow depressions in the integument over the regions where the true openings will ultimately be developed. The crabs collected at Wareham, in 1900, which were certainly not less than one year old, had the sexual ducts well developed (pl. 9, fig. 53, *sd*), and the sexual glands were large and complexly coiled, but not quite mature. This would mean that the production of sexual products would not have occurred in them before the following year, *i. e.*, the hermit crab is probably not mature before the second or third year of its life (see page 168).

Sex is recognizable at the seventh stage, from six to twelve days after the close of the glaucothoe phase, when the males lose the rudimentary pleopod on the second segment (pl. 4, fig. 6, *ru*). But the secondary sexual characters in the female pleopods (pl. 6, fig. 24) do not begin to appear until thirty or forty days of adolescent life have passed and then their development is quite gradual.

This early differentiation of sex in *Eupagurus longicarpus* and *annulipes* has an interesting bearing on the subject of parasitic castration as it exists in the allied *Eupagurus bernhardus*. There, Giard ('**86**) has found that the male crabs if infested with a Bopyrid, *Athelges paguri*, have pleopods of female number and form; while the females when parasitized with the Cirriped, Peltogaster, bear the typical female number of appendages, but in type these approximate those of the normal male. No data is at hand with respect to the adolescent development of *E. bernhardus*, but if it resembles that of our species at all closely, the modifications shown by the parasitized males require either the attachment of the parasite very early in adolescent life, or a sufficiently potent effect from its presence to cause a reappearance of the pleopod on the second segment. The alterations of the parasitized females on the other hand, would be explainable as arrest of development. The parasite presumably might attach itself at any time within the first fifty days of adolescent life and yet be able to check the complete development of the female type of appendage. No ecto-parasite has been found on our *Eupagurus annulipes*. The only ecto-parasite on *E. longicarpus*, a Bopyrid, *Stegophryxus hyptius*, produces no alteration in the secondary sexual characters of the host.

The circulatory systems of our various species of Eupagurus are similar in all, and that of *E. bernhardus* (Bouvier, '**91**) will serve as the type. The hepatic arteries are wholly thoracic, so that only a small part of the liver receives blood from this source. The ventral thoracic artery terminates posteriorly with the branches to the fifth pair of limbs and the abdomen is supplied by the superior abdominal alone. As this artery enters the second segment of the abdomen, it divides into two trunks: *b* and *b′*. The former is a small vessel which courses superficially to the left, supplying livers, sexual glands, and appendages. The latter is a larger trunk which plunges downward to the right of the intestine, runs caudad along the dorsal surface of the flexor muscles, and then in the fourth segment divides into a supramuscular branch that continues the course above the muscles, and an inframuscular branch that pierces them and runs caudad beneath them in the position of a ventral abdominal artery.

The blood vessels in the larvae can only be studied satisfactorily from serial sections and hence there are annoying gaps in my record. For the arteries are scarcely traceable unless distended with blood, and only a few specimens of any stage will chance to show the desired condition. As a rule, however, all the dorsal vessels were equally well preserved and much could be learned from a single specimen, but ventrally the shrinkage of the integument against the ganglia obliterated the arteries to a greater or less degree. These and other causes also, prevented a study of the venous sinuses.

The only artery passing cephalad from the heart in the earlier zoea stages is the anterior aorta, and it extends to the base of the rostrum, lying close beneath the dorsal wall of the cephalothorax. In the fourth stage its anterior end becomes deflected over the surface of the supra-oesophageal ganglion. No structure which suggested a cephalothoracic sac similar to that described for the zoea of Palaemonetes (Allen, '**93**) was found at any stage. A sternal artery is present at all stages and passes down in its adult relations to the thoracic ganglia, between the fiber masses for the third and fourth pairs of limbs (pl. 7, fig. 29, *st a*). The antennary arteries are first found in the fourth zoea and as they are not invariably present, they probably arise during the period. They diverge strongly and give off a branch to the stomach. Their ultimate distribution, however, could not be determined for the different stages. The hepatic arteries first appear during the glaucothoe stage after the livers have shifted to the abdomen. There is no trace of them in younger specimens even when the preservation of the heart and adjacent parts is perfect.

The deferred development of the hepatic arteries can scarcely be regarded as correlated with the reduced function of these vessels in the adult crab, although at first this might seem probable. For, although among Decapods it is usual (Claus, '**84**) for these arteries to be present throughout the larval period, this is not an invariable rule. The Thalassinid, Naushonia (M. T. Thompson, :**03**), has only antennary arteries and aorta forward from the heart during the zoea and mysis phases. Claus's figures in his monograph on the circulation in

Decapods show that in the Thalassinid, Calliaxis, and the shrimp, Crangon, the hepatic arteries, although appearing in the zoea phase, are yet later in development than the antennary arteries. Thus the latter in their development seem to precede the hepatics when both pairs are not present throughout larval life. And in Eupagurus the antennary arteries appear unusually late, not until the fourth zoea.

Unquestionably, the ventral thoracic artery does not enter the abdomen at any stage, but as already noted, this vessel was difficult to trace and the distribution of its branches could not be completely determined. It is certain, however, that in the first zoea, vessels pass off to the first maxilliped; that in the second zoea this artery supplies the third maxilliped and, in some cases at least, one or more of the rudimentary limbs; and in the fourth stage the artery extends to the mouth and gives off branches to the maxillipeds and to the five pairs of rudimentary limbs. It is only necessary to suppose that the artery gives off in addition to the branches already enumerated, vessels to the second maxilliped in the first, and to the two pairs of anterior maxillipeds in the second zoea, to make the arrangement for each stage agree exactly with the distribution of the vessels in the "Pagurus" zoeae studied by Claus ('84). The ascending arterioles which pass up through the nerve chain between the ganglia for the first and second limbs and in the next posterior interspace (Bouvier, '89, '91) are discernible as early as the second zoea. Two other ascending arterioles are found even in mature glaucothoe: one between the maxillipedal and the cheliped ganglia and one between the maxillipedal and the maxilla ganglia. They may yet be detected in the adult. Some sections of zoeae suggest the possibility of the existence during the earlier stages, of additional ascending arterioles between the individual maxillipedal and maxilla ganglia, but they are not conclusive.

The superior abdominal artery is present throughout the developmental period. With the earlier zoea it is a simple vessel extending almost the length of the abdomen in the first, and to the telson in the later stages. In the fourth zoea and glaucothoe, however, this artery gives off five pairs of segmental branches, a pair for each segment from the second to the sixth.

At the time when the livers commence their shift, the adult plan is evolved from this simpler arrangement (pl. 10, fig. 64). A new artery arises from the right segmental artery in the second segment, plunges downward toward the flexor muscles and during the last part of the glaucothoe period, pierces these. Its further development could not be followed, as it was impossible to interpret the blood vessels through sections of the adolescent stages, owing to the twist of the abdomen and consequent confusion of landmarks. But it is obvious that this new vessel is to be identified with *b′* of the adult crab's arteries and that *b* of the adult system is the superior abdominal. This latter artery swings to the left with the completion of the shift of the livers and as the displacement is usually accompanied by the suppression of the right segmental artery of the second segment distal to the origin of *b′*, the latter quickly assumes the adult relations and appears to arise directly from the superior abdominal, *b*. Occasionally, however, this segmental vessel will persist in mature glaucothoe and even into the sixth stage. The fate of the other segmental vessels is uncertain. The four posterior pairs can be identified in very mature glaucothoe and occasionally in sixth stage larvae. Their presence in the latter stage suggests that they may persist in the adult crab, but whether this is actually the case or not could not be determined. The anterior pleopods of the adult certainly receive blood from branches of the superior abdominal (Bouvier, **'91**). But on the other hand at this time this vessel does not extend to the rear of the abdomen and the uropods are supplied by the inframuscular branch of *b′*.

We have no data with regard to the abdominal arteries of any Pagurid outside of the genus Eupagurus and whether the peculiar abdominal blood system of this genus is generally distributed among the members of the group is not known. In case it is not generally present, it might furnish a valuable criterion of relationship. Among other Decapods the artery most nearly analogous to *b′* occurs in *Gebia deltura* (Bouvier, **'90**). This vessel, however, arises directly from the superior abdominal in the fifth segment and such a posterior position appears to me effectually to militate against regarding it as homologous with the Eupagurid artery, *b′*. The development of the latter suggests rather that it originated either as a new structure, or, more likely, as an enlargement of some minor branch of the segmental artery of the second segment, when the gradual suppression of the ventral abdominal artery necessitated a more perfect connection between the dorsal arterial trunk and the ventral region of the body. Once introduced, the branch has usurped many of the functions which ordinarily belong to a superior abdominal artery.

The abdominal musculature of the young Eupagurus was studied chiefly from serial sections. Although only longitudinal sections proved of value, this does not at all effect the accuracy of the results, since in examining the muscles of adult Cambarus or Homarus by means of sections, only those cut in the longitudinal plane are interpretable. Such sections, however, throw a great deal of light on the arrangement of the muscles in these Crustacea. And I feel the more confidence in my conclusions with regard to musculature in these forms from the fact that they are based on both dissections and sections, although they differ somewhat from those usually accepted. In any event, the abdominal muscles of the young Eupagurus are essentially like the muscles of Cambarus, Homarus, and other Macrura; and sections of each are mutually comparable (pl. 10, figs. 59, 60, 61).

The muscles of Eupagurus reach their highest efficiency with the glaucothoe stage. At this time the extensors are well developed with a generally longitudinal course (pl. 10, fig. 59, *ext*); the pleopodal muscles converge from a region of attachment above the hinge or metacleis where the segments interlock and are independent of the flexors (pl. 10, fig. 59a, *plm*). The flexors comprise several muscles, the descending, transverse, longitudinalis, and loop-enveloping. The arrangement of these various muscles can best be understood from the study of a single segment, selecting segment two as typical.

The abdomen of the glaucothoe is highly convex and hence the attachment above the hinge, the metacleis insertion, is more dorsal than in the flatter segment of Homarus. The descending and transverse muscles arise from this point. The former is a broad band whose ventral end is inserted at the articulation with segment one; the latter is cylindrical and runs first ventrally and then transversely to come into intimate union with its mate from the other side of the body. In Cambarus and Homarus the transversalis is flat and in the latter genus it arises as part of the loop-enveloping muscles.

The lateralis or lateral longitudinal passes from the anterior to the posterior borders of the segment at the sides; and ventrally above the nerve cord the ventralis or ventral longitudinal occupies a similar position. Possibly the latter is a series of muscles rather than a single bundle, but the sections are not clear on this point.

The loop-enveloping muscles, which form the bulk of the flexors, arise in common with the ventralis muscles from the anterior boundary of the segment, but ascend as a broad sheet of fibers (pl. 10, fig. 59b). They soon become transverse in course and gradually separate into two parts, the loop or circularis and the enveloping or oblique. The fibers composing the loop muscles, probably augmented by fibers from the metacleis insertion, turn longitudinally so that the anterior end of the muscle lies dorsad of the transversalis, meeting the posterior end of the loop muscle of segment one, while its posterior end passes into segment three and there meets the loop muscle of that segment above the transversalis. The middle portion of the muscle is depressed and the successive arcs are very characteristic in longitudinal sections. Toward the mid-plane of the body a slip (pl. 10, fig. 59e, x) passes down from the anterior end of the muscle to become attached at the union of segments three and four, but whether on the sternum of segment three or into the articular membrane cannot be determined. Still nearer the mid-plane, the whole muscle, the anterior fibers changing first, becomes a longitudinal descending muscle.

The fibers which go to form the enveloping muscle retain a more generally transverse course as they are separated from the fibers of the loop muscle; and therefore this muscle lies across the belly of the loop muscle (pl. 10, fig. 59d). Toward the mid-plane of the body it becomes in its turn descending, and goes down closely associated with the descending part of the loop muscles (pl. 10, fig. 59f, x_2). The attachments of the descending portions of these muscles are not clearly shown in the sections. At times they are apparently at the articulation between the segments three and four; again the muscles seem to pass on to the next posterior articulation, a discrepancy which is possibly due to an attachment in part at both points. The loop muscle is small in Cambarus and is largely or perhaps wholly derived from the metacleis insertion. In Homarus it is a large muscle and derived from the metacleis insertion and attached at its descending end to the sternum of the third segment. In Homarus the slip x is attached to the sternal plate of segment three. The enveloping muscle in both of these Macrura and in allied forms is very large.

The foregoing description for the second segment will apply equally well to segments one and three except that in the former the anterior attachments are on the cephalothoracic walls. The fourth segment has a weak loop muscle which barely reaches the end of the loop muscle from segment three. Posteriorly its descending portion seems to be attached at the articulation between segments five and six. The fifth segment has only pleopodal, transversalis, and ventralis muscles. The sixth segment has pleopodal, transversalis, and ventralis, and the last mentioned muscle lies beneath the nerve cord.

The muscles of the zoeae are on the whole similar to those of the glaucothoe, but less well developed and with weak attachments. The pleopodal muscles are wanting, except those for the uropods which come in with the fourth zoea. The three earlier zoeae have also the fourth segment like the fifth from the absence of loop-enveloping muscles.

Mature glaucothoe, adolescent crabs, and adults have a musculature of a totally different type. The extensors are extremely weak; a thin layer of fibers — the integumentary muscles — lines the integument beneath the nerve cord; the flexors are bulky and those of the right side are considerably larger than those on the left. But the flexors on both sides are merely a series of strongly diagonal bands with the more dorsal fibers running almost transversely. Minor peculiarities present themselves in the different segments, but there is nothing which suggests either transversalis or loop-enveloping muscles, although descending, lateralis, and ventralis muscles are doubtfully identifiable. These muscles have been compared to the chevron-like muscles of Gebia and Callianassa and described as a crowded series of such "chevrons" (Bordage, '93).

If these muscles are to be identified with their forerunners in the larvae, a study of their metamorphosis becomes imperative. But unfortunately this could not be attained in as complete a form as might be desired. The changes are crowded into a comparatively short period near the end of the glaucothoe phase, the degenerative processes make it difficult to secure good preservation and there seems to be a slight reconstruction or remodeling near the very end of the alterations. The following data are at hand, however, from several individuals, and from them a general notion of the homologies of the adult muscles can be obtained.

1. The transversalis muscles begin to lose their fibers at the time when the livers shift to the abdomen.

2. After the shift is completed and the nephrosac has passed to the abdomen, the loop muscle's fibers have become straight, and the descending portions of this and of the enveloping muscles are weak. The descending, lateralis, and ventralis muscles remain distinct, but the transversalis, the pleopodal muscles, and the intrinsic muscles of the pleopods themselves have disappeared.

3. Then the integumentary muscles appear; the descending and the ventralis still show fibers, but only a few fibers can be found in the loop-enveloping muscles. The columella prominence is developing.

4. Still later, traces of the enveloping and descending muscles can still be identified, but only with difficulty. The ventralis and integumentary muscles are somewhat more distinct.

The flexor muscles of the adult hermit crab then, evidently lack the transversalis elements, and retain only remnants either of descending or of lateralis muscles. The relative proportions of the remaining flexors, the ventralis and the loop-enveloping muscles, are not readily or surely determinable. Probably the ventralis plays the larger *rôle.*

The thin layer of integumentary muscles seems to be derived from scattered fibers that lie in the same position during the glaucothoe stage. These may also give rise to the apical fibers of the muscles in the columella prominence but the basal fibers of this organ are certainly derived from the ventralis of the third segment and it is possible that the others are also.

The theory that the flexor muscles of the hermit crab are a crowded series of "chevron" muscles is scarcely tenable in face of this evidence from the study of their structure and metamorphosis. The support that it has received from the chevron-like muscles of Gebia and Callianassa also fails when a closer examination is made. In Gebia certainly, the "chevrons" resolve themselves into loop-enveloping systems with a weak loop and insignificant transversalis, so that the preponderance of the enveloping element gives a notably oblique course to the muscles.

The larval hermit crab has, therefore, abdominal muscles more like those of generalized Macrurous Crustacea, than those of such Thalassinids as Gebia, and it would be interesting in this connection to know the type of musculature among the less specialized Thalassinoids. This might throw some light on the extent to which the perfection of the Macruran type in the Eupagurus larvae is palingenetic. For it is not possible to regard it as simply correlated with the active life of the zoea or glaucothoe. The very active megalops of the Brachyuran, Callinectes, although provided with enormous pleopodal and well developed descending muscles, has of the remaining possible flexors only the ventralis bundles; the metazoea of Pinnotheres and both the young zoea and the metazoea of Cancer show similar relations, with the addition of a few fibers which may be doubtfully identified as loop-enveloping elements. Moreover, Gebia, like other Macrura, has in the larval stages a very perfect loop-enveloping system of flexors, and these are carried on into the first adolescent almost intact, although the animal is at this period quite inactive.

The muscles of the stomach of the zoea and glaucothoe are very simple. At first only dorsal and ventral supporting muscles are present (pl. 9, fig. 47). But with the second stage two additional bundles arise with attachments near the point where the oesophageal plates will later appear and the third zoea adds two more which extend from the anterior face of the stomach forward to the cephalothoracic wall. This simple arrangement is retained by the metazoea and by the glaucothoe until the very close of the latter period. Then it rapidly gives place to the complex musculature of the adult. The new muscles are developed from myoblasts which lie around and above the stomach from the third zoea onward. Of the zoeal muscles, the pair that extend from the region of the oesophageal plate are alone retained.

It seems probable that the adductors of the mandibles in the adult are also new structures with the glaucothoe, and not derivatives of the fan of muscles that moves the jaws in the zoeae (pl. 9, fig. 49). But the moult from the fourth zoea produces so great a change in the plane of the mouth parts relative to the body axis that a satisfactory comparison between sections through this region in zoea and glaucothoe is impossible. The remaining muscles of the body call for no especial mention. The developing limbs remain filled with undifferentiated tissue until the fourth zoea and then fibers begin to make their appearance.

The thoracic portion of the nervous system of the adult hermit crab is symmetrical. The supra-oesophageal ganglion resembles in its finer structure the same ganglion in Carcinus (Bethe, '95, '97) or Astacus (Krieger, '80). The ventral ganglia are somewhat concentrated (pl. 7, fig. 29). Those that supply the mouth parts form a quadrate mass which is closely associated with the more posterior thoracic ganglia. The three interior of these are large with strong ventral projections of the fiber masses; the two posterior are insignificant.

The abdominal portion of the nervous system is but little modified by the asymmetry. The ganglion of the first segment is not apparent and is presumably fused with the thoracic mass (Bouvier, '89). The five remaining ganglia are distinct (pl. 7, fig. 30) and those for segments two, three, and four are displaced to the left. The displacement of the ganglion for the fourth segment is very slight and scarcely establishable in the adult crab, but is well shown in sections of adolescent crabs. Each abdominal ganglion gives off a pair of ganglionic nerves (*gn*) beneath the integumentary muscles, either to a pleopod or to the point where a pleopod morphologically ought to be situated. The areas supplied by these nerves also receive a more delicate commissural (*comm n*) above the muscles from the commissure cephalad from the ganglion. A similar arrangement of sub- and supramuscular nerves is found in *Gebia affinis*. There is no obvious difference in the size of the nerves supplying the right and left sides of the body, and they all show fibers and bipolar cells. This lack of asymmetry appears less striking when we recollect that these nerves are probably very largely sensory and this function would be nearly equal on both sides of the body through the hairs on the pleopods on the right and the tufts of hair on the left at the points where pleopods ought to stand. The function of the muscles also is very simple and so the larger size of the right flexors cannot greatly disturb the equality of the two sides of the body with respect to other structures.

The nervous system develops almost without metamorphosis. The earlier larval stages have a supra-oesophageal ganglion of essentially the same structure as that of the adult, but differing in the smaller relative bulk of the fiber masses and fiber tracts as compared with the ganglion cells. In the zoea phase these cells completely surround the fiber masses and tracts, and although less numerous in the glaucothoe, the cell groups still are indistinguishable and confluent. But by the time the adolescent stages are reached the groups though still united, become identifiable. The median ventral group, the cellulae mediales, is the first to become completely distinct and separate, in crabs forty days past the glaucothoe. The adult separation of all groups was found in the Wareham crabs. The globuli of the brain decrease in size in the earlier stages more rapidly than do the fiber masses. The diameter of each bears the following ratio to the width of the latter, measured in coronal or transverse sections at different periods: —

Stage	Diameter of globulus.		Width of intervening fiber mass.
Adult	1	:	1.42 +
Sixth stage	1	:	2
Fifth stage	1	:	2
Second stage	1	:	2.6
First stage	1	:	3

The infra-oesophageal ganglion has a history similar to that of the supra-oesophageal. The zoeae have small fiber masses which are deeply imbedded in ganglion cells and these latter are especially prominent on the ventral side of the ganglion. In surface view, therefore, the ganglia would exhibit as great a degree of concentration as in the adult, but in reality the individual fiber masses are much more distinct, especially those for the maxillipedal and maxilla segments. The fiber masses for the limb segments are very small in the zoea phase. With the glaucothoe, the cheliped ganglion alone shows a ventralward projection, this detail appearing for the other limbs in the adolescent phase. Nerves pass to the rudimentary limbs in the fourth zoea.

The three earlier zoea stages have possible traces of the first abdominal ganglion in two minute fiber masses that lie outside of the thoraco-abdominal commissures at their origin. Nothing of this can be detected later. The length of the segments of the abdomen is more nearly equal during the zoea and glaucothoe phases than during later life, and hence the five abdominal ganglia are nearly equidistant from one another at these periods. Both ganglionic and commissural nerves are present from the first zoea (pl. 7, fig. 29). At the close of the glaucothoe period, after the muscles are already much degenerated, the commissure between the ganglia of the second and third segments shortens by one third, the next posterior commissure shortens to a less extent, and commissures 4 and 5 lengthen. These changes bring the ganglia into their definitive distances from each other, accompanying the changes in the lengths of the respective abdominal segments preparatory to the moult to the sixth stage. The displacement of the anterior ganglia to the left is one of the last changes before the glaucothoe stage closes, and occurs as the columella prominence arises.

THE SHELL IN THE ONTOGENY.

Rathke noted in 1840 that the developing hermit crab became slightly asymmetrical before the close of the zoea phase and all observers have recorded asymmetry for the chelipeds and usually also, dissimilarity for the uropods in the glaucothoe phase. Agassiz ('75) went a step farther and found that a very considerable advance toward the adult asymmetry might be attained before the larva ever entered a shell. It seemed clear from his account that this could not be regarded as the invariable sequence; but how far it might be looked upon as typical remained uncertain. He says of the change: "When the moult has taken place which brings them to the stage when they need a shell [my sixth stage] we find an important change in the two pair of feet now changed to shorter feet capable of propelling the crab in and out of the shell; we find that all the abdominal appendages except those of the last joint are lost; but the great distinction .... is the curling of the abdomen; its rings .... are quite indistinct and the test covering it is reduced to a mere film. It is therefore natural that the young crab should seek shelter for this exposed portion of his body." His figures, published in 1882 (Faxon, '82), corrected and furnished a partial interpretation of this text. But they seemed to indicate that these changes might be only a beginning of a gradual metamorphosis to the complete adult form. It has therefore remained for the present research to show that the adult structure, so far as its plan is concerned, is completely attained during the glaucothoe stage, and that typically the metamorphosis is inaugurated before a shell is taken. Nevertheless the young crab always enters the shell before the changes are far advanced, usually within the first forty-eight hours of its life as a glaucothoe. What then is the significance of the shell in the ontogeny? It is clear that it cannot be regarded as an essential factor, but may it not perhaps influence the development to a lesser extent?

If such an influence were present it obviously might act in two directions: to affect the rate of the metamorphosis, or to modify the order of the metamorphosis and possibly, but less probably, the resulting structures themselves. In addition to this, despite the fact that the dextral asymmetry is fixed in the ontogeny, appearing before a shell is taken and completing itself even when the latter is absent, it is conceivable that the entrance into a dextral shell which normally occurs at a definite point in the development may have some controlling influence, so that exposure to other than dextral shells might not be without effect on the course or integrity of the metamorphosis.

Effect on the rate of development.— In connection with our first possible effect of the shell, that on the rate of the development, the influence, if any, will involve the glaucothoe more than a later stage as this is the period of most extensive change and the time when the shell is normally assumed. Therefore a number of experiments were performed on glaucothoe by which they were exposed to different environments relative to the time when the shell was taken and also to the shape of the shell, whether dextral, sinistral, or uncoiled.

For these experiments the age of the larvae used, had to be known within as narrow limits as possible. So fourth-stage zoeae were collected and placed in a large jar, where during the night many would moult to the glaucothoe. New zoeae were used each day, and in the morning the glaucothoe were removed and reared in one or more experiments as the case might be. For I made it a rule never to mix glaucothoe of different ages, but kept each batch separate from all preceding ones. The experiments were examined daily at the same morning hour. At first, an additional mid-period record was taken, but it was found that the larvae moulted chiefly in the "small hours" of the morning and this habit rendered a mid-period record less necessary and as the pressure of other work became severe it was abandoned. The fairly definite time of moulting also served to reduce the unavoidable time error which might otherwise have had a range of twelve hours. The large number of specimens used further counteracted this error and also tended to equalize it in the different series. So on the whole, the number of days that the larvae remained in the glaucothoe phase was determinable with a fair degree of accuracy, and although some error must remain, it does not affect the comparability of the series.

Series A.

Normal.

The glaucothoe were provided with dextrally spiral shells and given opportunity to take these whenever they pleased. The shells of the marine snail, Sycotypus, were used, being abundantly obtained from the egg cases. Eleven experiments were made, using 183 larvae, 99 of which attained the moult to the sixth stage.

6	glaucothoe	6	percent	remained	in	stage	4	days.
3	"	3	"	"	"	"	4½	"
57	"	57	"	"	"	"	5	"
2	"	2	"	"	"	"	5½	"
19	"	19	"	"	"	"	6	"
14	"	14	"	"	"	"	6½	"
4	"	4	"	"	"	"	7	"
2	"	2	"	"	"	"	7½	"
2	"	2	"	"	"	"	8	"

The percentages for the duration of the stage may be arranged in a curve in which the ordinates represent days, the abcissae the percentages (pl. 7, fig. 32, continuous line). This gives a very steep curve with the mode at the fifth day.

Series B.

Delayed Normal.

In these experiments, a batch of larvae was separated into two parts. One half was provided at once with dextral shells to serve as a control experiment; the other did not receive shells immediately. The figures for the control records have already been given as part of the figures for series A. They are separately listed here for comparison with the figures for the "delay" record.

B 1.

The larvae were kept three days without shells and were then provided with them, and they entered these within a few hours. One experiment, using 10 glaucothoe in the control section, all of which lived to the sixth stage; and 10 in the delay section, 7 of which attained the moult.

Control.				Delay.			
1 glau.	10 %	r'm'n'd	4 days.	1 glau.	14 %	r'm'n'd	5 days.
8 "	80 %	"	5 "	4 "	57 %	"	5½ "
1 "	10 %	"	5½ "	2 "	28 %	"	6 "

B 2.

The larvae were kept four days without shells and were then provided with them. Three experiments, using 55 crabs in the control, 25 of which attained the moult to the sixth stage; and about the same number in the delay, 22 of which reached that moult.

Control.				Delay.			
3 glau.	12 %	r'm'n'd	4 days.	6 glau.	27 %	r'm'n'd	5 days.
2 "	8 %	"	4½ "	2 "	9 %	"	5½ "
19 "	76 %	"	5 "	12 "	55 %	"	6 "
1 "	4 %	"	5½ "	2 "	9 %	"	7 "

The curves that may be constructed from the percentages of these experiments, resemble in shape the curves for the experiments of series A (pl. 7, fig. 32), but have a more restricted range. The curve for the three days' delay (broken line) has the mode between the fifth and sixth day; while that for the four days' delay (dotted line) has the mode at the sixth day. The curves for the control experiments exactly resemble the curve plotted for series A.

Series C.

Naked.

In these experiments, glaucothoe were reared without any covering for the body. Five experiments, using 123 larvae; 50 of which attained the moult to the sixth stage. No mid-period examination.

7 glaucothoe 14 percent remained in stage 4 days.
6 “ 12 “ “ “ “ 5 “
21 “ 42 “ “ “ “ 6 “
15 “ 30 “ “ “ “ 7 “
1 “ 2 “ “ “ “ 8 “

The curve that may be constructed (pl. 7, fig. 33, continuous line) has the same range as the curve for the experiments of series A, but its mode is on the sixth day and the seventh day percentage is high, giving it a very different shape from the curves for either A or B. It may be objected that the absence of a mid-period examination is responsible in large measure for this difference in shape. But the mid-period moults, if not separately recorded, will merely increase the record for the following morning and if the curve for A be plotted with the mid-period moults thus added to the day records (dotted line), it does not at all resemble the curve for the present series. So although the sixth and seventh day percentages may readily be somewhat higher than they would have been with a mid-period count, the difference would be insufficient to modify the curve essentially.

Series D.

Indifferent.

The glaucothoe were provided with straight tubes of such diameter that they could not twist the body. Glass tubes proved the best of the various objects tried. Three experiments, using 64 larvae, 39 of which reached the moult to the sixth stage. No mid-period examination.

35 glaucothoe 89.7 percent remained in stage 5 days.
4 “ 10 “ “ “ “ 6 “

The curve for these experiments would have a very limited range and show the mode on the fifth day.

Series E.

Sinistral.

The larvae were provided with small sinistral shells and given opportunity to take them at will. The shells of the fresh-water snail, *Physa heterostropha*, were used. Eight experiments, using 146 glaucothoe, 58 of which attained the moult to the sixth stage. No mid-period examination.

3	glaucothoe	5	percent	remained	in	stage	4	days.
35	"	60	"	"	"	"	5	"
12	"	20.7	"	"	"	"	6	"
8	"	13.8	"	"	"	"	7	"

The curve which may be plotted for this series is similar to the curves for A, B, or D except that the percentage for the sixth day is slightly higher (pl. 7, fig. 33, broken line). But this may be attributed to the fact that a large number of larvae, 36 percent, delayed their entrance into the shell and so retarded their development. The absence of a mid-period examination probably also contributes to increase the percentages for the sixth day.

These six series of experiments clearly show that the minimum duration of the glaucothoe stage and phase under any condition is four days, and the maximum duration is eight days. In all classes of experiments, except those of the "indifferent" and "delayed" series, some larvae remained in the stage the minimum period. The maximum period was reached not only in the "naked" but also in the "normal" series. The experiments also show that the shell exercises an influence on the rate of development proportional to the time which elapses between the moult from the last zoea stage and the time when the glaucothoe enters the shell. The experiments with shells of other than dextral coil, however, so closely resembled the latter "normal" experiments that no attempt was made to repeat them under "delay" conditions. They indicate that the influence of the shell is independent of its form as far as any direct effect is involved. But when shells depart strongly from the dextral type, an indirect influence is exerted, dependent on the fact that many larvae are reluctant to enter such shells.

Effect on health.—Another influence of the shell on the growing larva was brought out by the experiments. Attention has already been called to the high mortality among Eupagurus larvae. Now this mortality was especially noticeable in those experiments where the glaucothoe were either wholly prevented from entering a shell (C) or only permitted to enter one after a considerable delay (B 2). Here it reached about sixty percent. In the "normal" experiments and in the less abnormal experiments of the "delay" series (B 1), only forty-six and thirty percent respectively of the glaucothoe perished before the moult to the sixth stage could be reached. The entrance into a shell early in the glaucothoe phase has then an important bearing with regard to the health of the crab.

At first sight also this seems to be to some extent correlated with the form of the shell used. The glaucothoe that lived in straight glass tubes (D) lost thirty-nine percent of their number, a death rate less than that for the normally reared crabs. The crabs in sinistral shells, on the other hand, had a death rate as high as that prevailing among crabs which never obtained a shell. But of course, as before, this may be the result of the reluctance of glaucothoe to enter sinistral shells with the consequent prejudice to their health, rather than a direct effect of the peculiar twist of the shell. With my adolescent crabs the death rate among those reared with sinistral shells was not noticeably higher than among those reared in other kinds of houses. Although this is based on a smaller number of crabs, it would lend support to the idea that the higher death rate among "sinistrally reared" glaucothoe was indirect. A particularly disastrous experiment with adolescents showed a mortality among larvae living in dextral, indifferent, and sinistral shells, of fifty-seven, fifty-two, and sixty percent, respectively.

The absence of a habitation is invariably injurious. The mortality for "naked" adolescents in the above experiment was eighty-one percent. Even with wild adults death usually follows after a short continuance of the "naked" condition. Injury to the soft and defenseless abdomen is generally the immediate cause of death, but there are occasional indications which may point to some general physiological disturbance caused by the lack of a body covering.

Effect of the shell on structure.— Since the shell determines to some extent the duration of the glaucothoe phase and also affects the health of the crab, a further influence might be perhaps expected. But in vain. The order in which the different organs develop from the larval to the adult type is the same whether the glaucothoe obtains its shell early or late in the period. It is independent of the presence or absence of the shell, and is not affected by its shape. Moreover, no difference in the structure or arrangement of the parts of the body of sixth-stage crabs reared under the various conditions can be detected, except in the reduction of the pleopods. A crab of this age, typically, has no pleopods on the right side of the body, but this is not invariably the case even among "normally reared" larvae, and some crabs retain rudiments of one or more of these appendages. The metamorphosis of the pleopods has been expressed by the following formulae (see page 158): —

	Glaucothoe.	Sixth Stage.	Adult ♂.	Adult ♀.
Segment 1	O : O	O : O	O : O	O : O
" 2	R : R	Ru : O	O : O	R : O
" 3	R : R	R : O	R : O	R : O
" 4	R : R	R : O	R : O	R : O
" 5	R : R	R : O	R : O	R : O
" 6	+R : R—	+R : R—	+R : R—	+R : R—

However, since segments one and six do not vary, the formulae may be written in briefer form: glaucothoe = R, R, R, R : R, R, R, R; sixth stage = Ru, R, R, R : O, O, O, O; adult ♂ = O, R, R, R : O, O, O, O; and adult ♀ = R, R, R, R : O, O, O, O.

The nature of the variations from the normal sixth-stage formula under all conditions was studied in about five hundred crabs which had been reared from the glaucothoe as follows: 107 in dextral shells, 62 in straight shells, 103 in sinistral shells, and 221 without shells. Of these 493 crabs, 159 or thirty-two percent varied from the usual pleopod formula. The different variations were numerous, but they fall naturally into three groups: (a) those where one or more of the right appendages were retained as large (Ru +), medium (Ru), or small (Ru —) rudiments, in order from before backward; (b) those where there was loss in number or size of the pleopods on the left side; and (c) those where there was irregularity in the retention. The time that each crab was under the microscope had to be very limited and the dimensions given do not represent measurements, but are based on comparisons with the unaltered pleopods of the left side.

Class A.

Rudiments without joints or rami. Twenty-five combinations, but only a few common to several specimens, *viz.*: —

Ru,	R, R, R : Ru,	O,	O,	O,	9	examples.	All series.
Ru,	R, R, R : Ru,	Ru,	O,	O,	8	"	All series excepting indifferent.
Ru,	R, R, R : Ru,	Ru,	Ru,	Ru—,	12	"	Naked series only.
Ru,	R, R, R : Ru,	Ru,	Ru,	Ru,	14	"	All series.
Ru+,	R, R, R : Ru+,	Ru+,	Ru+,	Ru+,	13	"	Naked and indifferent only.

Class B.

Rudiments without joints or rami. The variations of this class are probably only the result of defective ecdysis or injury during the glaucothoe stage. Three examples.

Ru+, Ru+, Ru+, Ru+ : Ru,	Ru,	Ru,	Ru,	1	example.	Normal series.
O, R, R, R : O,	O,	O,	Ru,	1	"	Sinistral series.
Ru+, Ru+, Ru+, Ru+ : Ru+,	Ru+,	Ru,	Ru,	1	"	Indifferent series.

Class C.

Rudiments without joints or rami except once, where the appendage was small, but perfectly formed and of male type (R*). Eleven examples in all.

Ru, R, R, R : Ru,	O,	Ru,	Ru,	1	example.	Normal series.
Ru, R, R, R : Ru,	Ru,	R,	O,	1	"	" "
Ru, R, R, R : O,	Ru+,	O,	O,	1	"	" "
Ru, R, R, R : Ru,	O,	Ru—,	O,	1	"	Sinistral series.
Ru, R, R, R : O,	O,	Ru,	O,	1	"	" "
Ru, R, R, R : O,	Ru—,	Ru—,	O,	3	"	Naked and sinistral series.
Ru, R, R, R : Ru,	Ru+,	Ru,	O,	1	"	Naked series.
Ru, R, R, R : O,	O,	R*,	O,	1	"	Sinistral series.
Ru, R, R, R : O,	Ru+,	Ru,	R,	1	"	Naked series.

The distribution of the variations.— The crabs that showed the variations, the *variants*, were distributed among the different conditions as follows: —

1.

Normal	107 crabs	21 variants	19.6 percent.
Indifferent	62 “	10 “	16.0 “
Sinistral	103 “	23 “	22.2 “
Naked	221 “	105 “	47.0 “

The percentages of the actual combinations under each environment to the whole number of variations recorded for all environments were: —

2.

Normal	28	percent	of	all	the	combinations.
Indifferent	26	“	“	“	“	“
Sinistral	36	“	“	“	“	“
Naked	221	“	“	“	“	“

The actual combinations of each kind presented, as compared with all the recorded combinations for each class of variations were: —

3.

	A	B	C
Normal	24 %	33 %	30 %
Indifferent	32 “	33 “	—
Sinistral	36 “	33 “	50 “
Naked	94 “	—	25 “

The distribution of the variant crabs among the classes of variations was as follows: —

4.

		A	B	C
Normal	21 variants	86 %	4.7 %	14.0 %
Indifferent	10 “	80 “	4.7 “	—
Sinistral	23 “	74 “	4.7 “	17.0 “
Naked	105 “	96 “	—	3.8 “

The first two tables closely parallel the results of the experiments on the rate of development. Here again the emphasis is placed on the actual presence or absence of the body covering rather than on its form. The crabs reared in sinistral shells had only a slightly larger percentage of varying individuals or variants, and only a little higher degree of variability than normally reared larvae. But those which had never entered a shell showed more than twice the normal percentage both of variants and of variability. The crabs reared in straight shells were less variable both in respect to the number of variants and to the combinations of characters than the normal, just as in the earlier experiments nearly ninety percent of the "indifferent" glaucothoe remained five days in the stage as against fifty-seven percent of the normally reared glaucothoe. I cannot suggest any explanation for this phenomenon.

Tables 3 and 4 are somewhat disappointing. The greater variability of the "sinistral" and "naked" crabs over normally reared, is not, except with the variations of class A, either united with a tendency toward a larger percentage of combinations compared to all the combinations for any class, or with leanings toward the peculiar variations of class C, although, *a priori*, both contingencies might have been expected. The numerous variant crabs of the "naked" series were almost all members of class A. Three of the most striking variations found, *viz.*: Ru, R, R, R : Ru, R*, Ru, O, Ru, R, R, R : Ru, Ru, R, O and Ru, R, R, R : O, Ru+, Ru, R, were presented by crabs from the normal, sinistral and naked series.

Although the presence of a sinistral shell or the lack of any covering for the body affects the larva only with respect to one series of organs, the pleopods, it might seem that a continuance of such a condition might be more potent; at least to conserve the variations already present. The rudimentary pleopod on the second segment becomes in the female the largest of the abdominal appendages. May it not be possible for a similar reconstructive power occasionally to reside in those other rudiments which are retained by so many sixth-stage larvae, despite the fact that typically with the males this appendage lacks reconstructive power and is lost very early in adolescent life. On experiment, however, it was found that outside of the pleopods no organ was affected at all by a continuance of abnormal conditions and only in a single case were the latter altered.

In the summer of 1900, a number of crabs from "sinistral" experiments were kept with sinistral shells. No especial attention was paid them. One of these grew much more rapidly than its mates and when it was about eighty days past the glaucothoe phase it was examined. It then had a carapace 3.7 mm. in length, an antennal flagellum of 35 joints, an ophthalmic scale which was adult in form, and sexual "pseudo-orifices" on the third pair of limbs, *i. e.*, in the female position. The pleopods, however, were of male number and type with the addition on the right side of the fourth segment of a pleopod exactly like the corresponding appendage on the left. The formula was: O, R, R, R,: O, O, R, O.

I am not inclined to attach much importance to this peculiar specimen and least of all to regard its peculiarities as connected with its life in a sinistral shell. With the exception of the pleopod it was internally and externally, a dextrally asymmetrical crab. Although it had lived for eighty days from the glaucothoe, it had not lost the male type of pleopod, when normally the female type should be almost or completely established. Moreover, the pleopods were of male number, while the "pseudo-orifices" were in the female position. It seems to me that this crab was merely a sport, resulting very probably from the unnatural conditions of captivity, and just as likely to have appeared in one series of experiments as in another. However, it certainly shows that the retained rudiments of the sixth stage may occasionally be conserved or reconstructed.

An attempt, during the following year, to repeat this experiment with larvae that were known to be variants, was unfortunately rendered inconclusive by an unusually high mortality. One hundred and eighteen variant sixth-stage crabs were collected, chiefly from "naked" and "sinistral" experiments, and reared to the adult form under the following conditions: 30 in dextral shells, 7 in straight tubes, 30 in sinistral shells, and 58 without shells. Out of these only 37 survived to be examined — a dead crab is almost immediately so mangled by the survivors as to be worthless for study — and in no case did any crab preserve the variations beyond the seventh stage and only 16 percent retained them into that stage. That is, whenever the moult from the sixth stage failed to give the normal adult pleopod formula, the succeeding ecdysis produced it.

Further investigations ought to be made in this direction. They require but little care outside of a supply of food, as metazoea may daily be placed in a jar, the glaucothoe removed as they appear and reared in balanced aquaria, new larvae being continually added and the young crabs studied as fast as they attain the adult form. Reliance should be mainly placed on sinistral shells. It is a waste of material to keep adolescent crabs naked.

The results from the foregoing study of larvae under various conditions with respect to the shells, effectually dispose of any probability that the adult crab can be modified by altering the form of the shell in which it lives. If in the presumably most plastic period of the animal's life, when there is in addition a slight variability to work upon, we are unable to alter the structure except in a most trivial detail and then but rarely — supposing that the retention of the pleopod by the crab in 1900 was due to the form of the shell — we cannot expect for an instant that even a long continuance in an abnormal shell will modify the adult.

The occasional discovery of a hermit crab (Milne-Edwards & Bouvier, '**91**; Marchal, '**91**) in a left-handed shell has no significance in this connection. The supply of shells runs, along shore at least, very close to the demand. Residence in a sinistral shell simply means that the crab has been dispossessed and is protecting his body with the best hollow object available. An endless list of such habitations might be compiled. I have collected adult crabs in the shells of Crepidula and Vermetus; and my glaucothoe used every hollow object they came across, from bits of algae that were twisted or rolled and broken float-bladders of Fucus or Sargassum, to fragments of exuviated crustacean integument and fragments of the fine dirt-tubes of small Annelids. There is no case where any departure from the normal form has been found in a crab which was using an unusual residence.

Choice of shells. — A consideration of the dextral asymmetry of our hermit crabs with the *rôle* played by the shell in the ontogeny, naturally leads to the question whether these crabs evince any preference for one type of shell over another. The only investigator who has attempted to study this experimentally (Bouvier, '**92**) has come to the conclusion that they do not show a preference for dextral over sinistral shells. He experimented by supplying the crabs with a mixture of shells of different sorts and then keeping a record of their movements. Approached from this side, the problem is difficult of solution. When a crab seizes on a new shell, it turns it over and over, thrusts the chelipeds within the chamber and then probably enters it. As far as I can judge, the preliminary exploration merely tells the crab that the "shell" is hollow, empty, and clean, and I cannot agree with those who would see in it a measuring or comparing of the shell. Apparently the crab does not perceive either the type or the size of the shell until it has inserted its abdomen into the chamber and tested the shell by moving about in it, etc., deciding by actual trial whether the new will prove better than the old house. It almost invariably keeps a firm hold on its former habitation, so as to be able to return to it if the new house proves ineligible. This method of selection by trial leads to numerous changes that are inconclusive and only serve to confuse an observer.

On the other hand, there seems to me to be evidence which points to a preference for shells of dextral type. If a single right-handed shell is dropped into an aquarium where the crabs are in straight tubes or sinistral shells, it will in the long run be found and used — unless its size be utterly inadequate — even though the bottom is encumbered with empty shells of other sorts. Similarly there is a tendency to abandon sinistral shells for straight tubes. This does not indicate any deliberate choice. The crabs are constantly changing shells and if one obtains the dextral shell he does not as readily exchange it as he would a shell of other type. Hence that shell will be found in use when the aquarium is examined.

In general, for both larvae and adults, the need seems to be, first for a covering and then for one of suitable size and shape. The animal is twisted dextrally. Therefore, other things being equal, the desire for a well fitting house will tend to bring the crab into a right-handed shell. But there are evidently a number of unknown factors which enter into a choice, especially a choice between shells of the same type. And there are many instances where crabs leave shells that, as far as appearances go, are vastly more suitable than the ones they substitute for them.

The larvae show the tendency to take dextral shells more strongly than do the adult crabs. The experiments show that young provided with shells of unusual form are more restless than those provided with dextral shells. The number that were found swimming after the first twenty-four hours, which means that they were either deferring their entrance into a shell or had come out again after entrance, aggregated 36 percent of all individuals in the sinistral series, 20 percent for the "indifferent" and 16 percent in the normal series. The observed cases where glaucothoe abandoned their shells when disturbed, were 7 percent in the sinistral experiments, but only 1 percent in the normal. In the "indifferent" experiments no specimen was observed to act in this manner. Moreover, a single dextral shell dropped into an aquarium will certainly be found and used.

When several glaucothoe are placed in a dish on the bottom of which there are scattered various shells, we find that as each larva stops swimming, settles down and crawls on the bottom, it examines the objects in its path and is apt to enter the first that has a cavity in it. Then, if not satisfied, it either recommences swimming or, more usually, makes a further search dragging about its newly acquired house. It is well known (Agassiz, '75) that in the investigation and use of a shell the glaucothoe handle the shell exactly in the same manner as do adults. My observations would make them subject also to the same limitations.

Like other investigators, I have not been able to repeat the observation (Brooks, '99) that hermit crabs "even make vacant [a shell] that seems eligible by pulling out its occupant piece by piece and eating him," although a dead snail is quickly devoured by the crabs and the emptied shell not infrequently used. If the snail, while the crabs are pulling and picking at it, gets pulled out bodily, the vacant shell may indeed be taken by one of the diners, but more usually the crabs devote their attention to the meat and leave the shell unnoticed. I have never seen anything which would imply an intentional or necessary connection between the eating of the snail and the use of the vacant shell. When, however, a crab is searching for a shell he acts in a very different manner, attempting to pull out the dead meat bodily, just as other rubbish is removed from a shell that is being examined. The snail may be eaten after removal, and I have seen a shell-hunting crab eat fragments which were torn off while he was trying to extract the snail. But this is unusual; for, as a rule, if the snail does not come out readily the crab either abandons the attempt, or if desperately in need of a residence crowds in between the meat and the shell.

The Asymmetry.

Although it is evident that the structural modifications possessed by the majority of the hermit crabs are on the whole in closest correlation with the use of dextrally spiral shells as residences, there are two genera in the group which suggest that the asymmetry in its beginnings was perhaps not connected with this mode of life. Mixtopagurus certainly might be regarded as pointing to an origin for the asymmetry prior to, or outside of the use of a coiled shell, as it combines a slight dextral twist with residence in holes in bits of wood. The sinistral asymmetry of Henderson's genus Paguropsis also seems difficult of explanation on any theory which would derive the asymmetry primarily from the use of shells. For the right-handed spiral has always been the predominant type of coil among the marine Gastropod Mollusca. The point of view with regard to these genera remains unchanged whether the Pagurids be considered as a natural or a convergent group.

This question of the origin of the asymmetry seems to me to be insoluble at the present day. Detail of the adult anatomy is as yet very scanty and for those genera about which most is known it does not bear closely on the problem. Probably a large number of the anatomical peculiarities of such a typically asymmetrical genus as Eupagurus, for example, may be looked upon as inherited; similar structures being found among the Thalassinidea, the modern representatives of the stock from which the hermit crabs were in all likelihood derived (Ortmann, :01). For example, the weak integument of the abdomen finds its counterpart among the members of the latter group. These live in burrows or crevices and probably their ancestors had similar habits. So the Pagurids might have attained a much weakened integument before they began to exchange stationary for movable "burrows." There is a curious resemblance between the degenerative details of the abdominal muscles of the Eupagurids and those of Gebia. Unfortunately, knowledge of the abdominal musculature of more generalized Thalassinids than Gebia is wanting. And even if the details shall be found to agree in the two groups, such a reduction of parts may be merely the usual outcome where the abdomen is little used by a burrowing species and not necessarily peculiar to the Pagurids, nor inherited from their Thalassinoid ancestors.

Other anatomical details also fail to throw light on the problem. The alterations by which the posterior end of the body has been transformed into a hook-like organ point to life in a movable residence, but might have been developed in either a straight or a spiral house. The tuberculated areas on the uropods in the more symmetrical hermit crabs and on both uropods and posterior thoracic feet in the asymmetrical forms, do not even indicate life in a movable residence. They are found in Pylocheles, which lives in the cavities of sponges. The reduction of the ventral abdominal artery also may have come in either early or late in the development of the hermit crab group. This vessel is very weak in some Thalassinids, *e. g.*, *Gebia deltura* (Bouvier, '90), and is still present in the anterior abdomen of so asymmetrical a Pagurid as Paguristes (Bouvier, '90a). The record might be thus continued with respect to other characters and with the same result. Our knowledge of the adult anatomy is insufficient to throw light upon the origin of the asymmetry, although, as already noted, the anatomy has been profoundly modified for the life in spiral shells.

The metamorphosis is also inconclusive on this point except in one particular; and this merely points to an early use of a dextrally spiral shell and does not bear upon the beginnings of the asymmetry. I refer to the displacement of the right liver to the left of the intestine and mid-line of the abdomen as the livers pass back from the thoracic position. That this must be interpreted as palingenetic seems to me to be fairly certain. Not only is it natural to extend this significance from the liver shift itself to the displacement, but the change also is an essentially transitory phenomenon and it is very hard to imagine any cause on other grounds for its introduction into the metamorphosis. If the displacement be palingenetic, it will go far to support the idea of an early use of shells. The shift of the livers in the phylogeny of the group probably occurred very early, for these organs have had time to attain a complicated structure since reaching the abdominal region. Further, they lie far back in the thorax in modern Thalassinids. Now, if these organs were already abdominal at the time when the shell was first assumed, it is difficult to understand how the compression of the right side of the body against the columella of the shell could have caused a displacement rather than an atrophy of the right liver. But if they were still thoracic or barely abdominal when life in shells first began, displacement during the development of the abdominal position would be the most likely outcome. These considerations make it seem probable that the shell was taken early in the history of the group, and so indirectly support that theory which derives the asymmetry primarily from use of shells.

Another important consideration adds further support to this view. At the time when the ancestor or ancestors of the hermit crabs began to seek other residences than burrows or crevices, the chances would have been favorable for the immediate use of dextrally spiral shells. The Pagurid group cannot have originated before the late Cretaceous or early Eocene (Ortmann, :01) while the Gastropoda are geologically a very old group. So we may suppose that a supply of mainly dextral shells would have been at hand when the hermit crabs began to use "movable burrows" and the asymmetry might have been affected by the shell at the very outset. This of course leaves the sinistral asymmetry of Paguropsis unexplained.

Thus the whole question falls back on general considerations, and on such a basis there is no reason to abandon our "shell" theory despite the lack of positive evidence. The relationships of such forms as Mixtopagurus and Paguropsis to the other genera of the group are wholly unknown. In fact, the relationships existing between the various genera as a whole, are very obscure. The group is in all likelihood convergent and not natural, although there is reason to believe that most of the genera are genetically related. If convergent, the parts of the group may be of different ages, the characteristic mode of life being assumed at different times by various forms. All this confusion must be cleared away before the asymmetry can be thoroughly understood. And this will require a vastly more extensive anatomical knowledge than we at present possess. The extent of the group and the poor preservation of the internal anatomy, particularly in the abdominal region, have hitherto turned investigation toward description of species and genera. While this is necessary, it usually does not lay enough emphasis upon the anatomy as a whole, but is content with a record of the more obvious and superficial detail of carapace and limbs. Now research must go farther afield. We need information with respect to the finer details of the structure in each species. I believe the clue to the origin of the asymmetry and to the phylogeny of this group of Crustacea lies in a study of the internal rather than the external anatomy.

The Glaucothoes.

In the year 1830, Milne-Edwards described a small, supposedly adult shrimp under the name "*Glaucothoe peronii.*" No one seems to have questioned the validity of this form as a true species, until the year 1864, when Müller pointed out its similarity to the postzoeal stage of "Pagurus" and suggested that it might be a larva. But since that time the problems arising out of Müller's suggestion have attracted the attention of several carcinologists. On the one hand, Bate (**'66**, **'68**) asserted that Glaucothoe was "only a larval Pagurus" and Faxon (**'82**) simply identified his postzoeal Pagurids with Milne-Edwards's genus and with the older genus Prophylax (Latreille, **'30**); while on the other hand, Claus (**'76**) asserted that the structure of the mouth parts in Glaucothoe was adult rather than larval; Miers (**'81**) described a second species, *G. rostrata*; and Henderson (**'88**) described a third species, *G. carinata.*

More recently the whole problem has been re-attacked by Bouvier through a study of the Glaucothoes themselves and, in the year 1891, he published the results of his work. He concluded that these forms were undoubtedly larval, since they lacked sexual orifices and opthalmic scales; that they were Pagurid rather than Thalassinid and belonged to the asymmetrical Pagurids; that they were not a natural group, for on structural grounds *G. peronii* was assigned to Sympagurus, while *G. carinata* lay nearer "Pagurus or a related form"; and that they presented "exactly all the essential characters of the larvae described by certain embryologists under the name of glaucothoes."

Now my research, as has been the case with the work of other students of Pagurid development, does not bear directly on the main problem presented by these peculiar crustacean forms. But it does involve some subordinate phases of the problem and affects some of the suggestions which have been put forward during the discussion. Two main objections have been brought against the "larval theory": the great size — 10 to 20 mm.— of the Glaucothoe as compared with all known glaucothoe-stage larvae, and the feeling that if these forms were really developmental stages they would be more abundant. Henderson (**'88**) has especially laid stress on this latter point. It seems to me that the cogency of both of these objections has been overestimated.

Although historical parallels are unsafe, it is interesting to note the essential likeness of the argument from relative size to one of the objections brought by Westwood ('35) against J. V. Thompson's assertion that the higher Decapoda developed through a metamorphosis. He said that he had collected "specimens of zoea that were ten lines long between the spines, ... far too large to suppose that they would subsequently put off their zoe form and appear as crabs, bearing at the same time in mind .... the minute size of the latter animals in the very young state, although possessing their ordinary form."

Similarly, the supposed rarity of the Glaucothoe is more apparent than real. When we remember that these forms were almost all collected at some depth below the surface, the number already taken — about twenty — does not seem especially small. And further, even among littoral Crustacea the larvae are often less abundant than would be inferred from the prevalence of the adults. The young of the lobster, Homarus, for example, swim freely for ten to twenty days after they hatch, but they are not commonly taken in tow nets. The glaucothoe larvae of the Pagurids themselves have seldom been recorded. The Plankton expedition of 1889 does not record a Pagurid zoea, and the glaucothoe phase is relatively much rarer than the zoeae. At Woods Hole, where hermit crabs are very abundant and the zoeae are obtained in unusually large numbers, fifteen or twenty glaucothoe would be an excellent haul for one day, and the average would be much lower than this without considering the days when the animal plankton is extremely scanty, although occasionally I have collected, on particularly favorable night tides, when there was a full moon, nearly or over a hundred glaucothoe.

My research bears directly upon Bate's suggestion ('68) that glaucothoe-phase larvae probably continue in the form until they obtain a shell and perhaps moult and grow. Recently this theory has been elaborated by Bouvier ('91a) as directly applied to the Glaucothoes themselves, in explanation of their size and rarity. "They are, so to speak, less fortunate larvae than the rest, which continue to grow until the time that they find a suitable habitation." Now, I find in the genus Eupagurus a glaucothoe phase that comprises only one stage, during which there is almost no increase in size. The duration of this period is short and it is but little affected by delay in obtaining a habitation. These results are found to be fully in accord with Agassiz's earlier observations ('**75**) on the same genus, when his rather obscure note is critically examined. And they ought to make us cautious in assuming as at all characteristic of Pagurid ontogeny either the existence of more than one stage in the glaucothoe phase or growth during that period.

However, the multiform and possibly polyphyletic nature of the Pagurid group must not be overlooked. So that no general conclusion can safely be based on the ontogeny of a single genus. Especially will this be true of the Glaucothoes. Despite Bouvier's conclusions, there seems reason to question whether they can be the young of forms closely related to those genera for which glaucothoe-stage larvae have been described, *viz.*: Eupagurus (Rathke, '**42**; Bate, '**68**; Faxon, '**82**; Sars, '**89**), Spiropagurus (Sars, '**89**), and Diogenes (Czerniavsky, '**84**). The mouth parts of Glaucothoe as figured by Milne-Edwards appear more mature in character than the corresponding appendages in the glaucothoe phases of *Eupagurus anullipes*, *longicarpus*, or our variety of *bernhardus*. For the equivalent stage of the European *E. bernhardus* we have figures of the maxillipeds alone (Rathke, '**42**), but they agree in all respects with the same appendages in the New England species. The mouth parts of the other described glaucothoe larvae have not been studied. The differences in the mandibular palp, in the first maxilla, and anterior maxilliped are especially noticeable in this connection, for in Eupagurus these parts have retrogressed from their condition in the zoeae and are without setae or at most only provided with rudimentary setae, while in Glaucothoe they are at least setose.

The absence of ophthalmic scales also is in contrast to the conditions existing in the larvae studied by Sars ('**89**) and Czerniavsky ('**84**) and probably those examined by Rathke. With respect to his larvae Rathke ('**40**, '**42**) only makes the indefinite statement that the eyes had the same form as those of the adult, but he seems to have worked upon the same species as did Sars. Of Bate's larva ('**68**) nothing is known with respect to these structures, and the young figured by Faxon ('**82**) do not have the ophthalmic scales. This last is almost unquestionably attributable to oversight. The figure was drawn from a rough sketch in which there was no intent to show more than the general form. Moreover, if the figure is correct with respect to the scales, it must represent the glaucothoe phase of some other genus than Eupagurus. Four species of this genus have these scales in the glaucothoe stage. As the figure was drawn from a specimen collected near Woods Hole, a region characterized by the practically exclusive predominance of the genus Eupagurus this would be a most unlikely contingency.

At the present time three "species" of Glaucothoe are recognized, but Bouvier (**'91**) has noted that the specimens of *G. peronii* collected by the "Talisman" are not wholly like Milne-Edwards' type specimen. If as I believe, the glaucothoe phase in the crabs from which these larvae are derived, is short and without any appreciable growth, these discrepancies can no longer be explained as differences in age and development. They indicate rather that the young of more than one species of hermit crab have been grouped under the single name.

SUMMARY.

The adult Eupagurus has a dextral asymmetry which not only affects every organ in the abdomen but extends into the thoracic region, so that scarcely any system of organs in the body escapes some modification or displacement. However, with the exception of the flexor muscles and arteries of the abdomen, the homologies with corresponding parts in other Decapoda are clear. But the diagonal muscle bands and the peculiar division of the superior abdominal artery into the two trunks, *b* and *b′*, are interpretable only from a study of the larva. The muscles are then shown to be a greatly degenerated loop-enveloping system from which the transversalis muscle has been lost. The arteries resolve themselves into supra-abdominal and a new vessel, primarily derived from the second segmental artery of the right side. This latter artery is probably peculiar to the Pagurids and without homologue among the higher Macrura.

The development is concentrated. There are four stages in the zoea phase, the last of which is a metazoea. The postzoeal or glaucothoe phase consists of one stage which is Macruran in general form and from the first presents a mingling of adult and larval characters. The external anatomy, especially of the cephalothoracic region, recalls the adult. The appendages — except the pleopods — the gill formula, the asymmetry of the chelipeds and uropods, the structure of the stomach, the concentration of the nerve ganglia

and the absence of a distinguishable nerve center for the first abdominal segment are more adult than larval. On the other hand, the anatomy and positions of the livers, and lateral caeca, the musculature of the stomach, the lack of an intestinal caecum, and the great elongation of the achitinous portion of the gut, the position of the sexual cells beneath the pericardial septum, and the preponderance of ganglion cells over fiber masses in the nervous system, are essentially as in the zoeae. Also many organs may be interpreted as presenting palingenetic as well as developmental characters. The cephalic position of the green glands, the thoracic position of the livers, the structure of the abdominal muscles and arteries, the complete set of pleopods, and the segmented abdomen may possibly serve as examples of this.

The metamorphosis by which the structures attain the adult type of arrangement, commences before a shell is taken, although the larva usually enters a shell within the first forty-eight hours after the moult from the zoea phase. Through it, the livers, sexual cells and green glands become wholly or in part abdominal, the arterial system in this region is reorganized, and its muscles and pleopods degenerate. The anatomy becomes completely adult *in type* before the glaucothoe period ends.

The stimulus of a shell is not necessary for the completion of this metamorphosis, any more than for its inauguration. The glaucothoe that has never entered a shell attains the adult structural type exactly like the glaucothoe which may have taken a shell immediately after the moult from the zoea phase. The shell is, nevertheless, very important. The length of the glaucothoe period bears a direct relation to the time that elapses between the moult from the zoea phase and the entrance into a shell. Under normal conditions the period ranges from four to eight days, lasting five days for a majority of individuals; but deferring or preventing the taking of a covering for the body prolongs it, so that it may reach six or seven days for a majority of individuals. The range, however, is not altered. This effect is derived from the presence or absence of the shell, and is not dependent, except indirectly, on any peculiarities of its form.

The shell also deeply affects the health of both glaucothoe and adult. Among glaucothoe which do not obtain a covering for the body or only obtain one after a delay of some days, the death rate is higher than among more normally reared individuals. The form of the shell here plays only a very subordinate part. Adolescent crabs which are kept without shells show an increase of mortality similar to that among glaucothoe.

The anatomical modifications that appear during the glaucothoe stage are, with but one exception, uninfluenced by either the presence, absence, or form of the shell. The exception is found in the retention of rudimentary pleopods on the right side of the body in the sixth stage, though typically at this period appendages should be absent from this side. The percentages of crabs that vary from the typical arrangement and the number of the variations displayed, are larger among larvae which have been reared without shells. The form of the shell is important to a small degree, and sixth-stage larvae reared from the glaucothoe in sinistral shells are slightly more variable than individuals reared with dextral shells.

In a single instance a crab reared in a sinistral shell reconstructed or retained one perfect pleopod on the right side of the abdomen. But this crab was otherwise so abnormal that there is reason to doubt whether the variation was connected with the special environment. Outside of this case, there is no evidence that the anatomy can be modified by a longer or shorter residence in shells of peculiar shape. Typically the retained pleopod rudiments are lost in the moult that closes the sixth stage.

There seems to be no reason to infer that hermit crabs learn the shape or size of a shell, except by trial and use. But on the other hand there is evidence for the assertion that they show a preference for dextral shells over those of other forms, based on the fact that if a dextral shell is dropped into an aquarium it will, in the long run, be found and used, even when the bottom is encumbered with shells of other kinds.

It is not possible at present to determine whether the asymmetry of the Pagurids is primarily attributable to life in spiral shells. The relationships of the genera are too imperfectly understood. Nevertheless, the asymmetry in its structural details is very closely adapted to the conditions imposed by this mode of life, which raises a strong presumption in favor of the view that the asymmetry was, from the first, a result of life in dextrally spiral shells. If the displacement in ontogeny, of the right liver to the left of the intestine points to a similar displacement in phylogeny the latter might well be the outcome of a compression of the right side of the body at the time when the livers were passing from the thoracic to the abdominal position such as a shell would cause. There is reason also to believe that this shift took place early in the history of the Pagurid group. Finally, the chances that the ancestral hermit crabs would use dextral shells rather than other objects at the time when they began to seek movable residences, are rendered very great by the age of the Gastropod Mollusca as a group, while the Pagurids are of comparatively recent origin.

LITERATURE.

Agassiz, A.

'75. Instinct ? in hermit crabs. Amer. journ. sci., series 3, vol. 10, p. 290–291.

Allen, E. J.

'93. Nephridia and body-cavity of some Decapod Crustacea. Quart. journ. microscop. sci., new series, vol. 34, p. 403–426, pl. 36–38.

Bate, C. S.

'50. Notes on Crustacea. Ann. and mag. nat. hist., series 2, vol. 6, p. 109–111, pl. 7.

'66. Carcinological gleanings.— No. 2. Ann. and mag. nat. hist., series 3, vol. 17, p. 24–31, pl. 2.

(Preliminary note for next article.)

'68. Carcinological gleanings.—No. 4. Ann. and mag. nat. hist., series 4, vol. 2, p. 112–121, pl. 9–11.

(Two zoeae and the glaucothoe of "Pagurus.")

'76. On the development of the crustacean embryo, and the variations of form exhibited in the larvae of 38 genera of Podophthalmia. Proc. royal soc. London, vol. 24, p. 375–379.

(A list of species whose zoeae were seen by W. H. Powers. He asserts that the young of *Birgus latro* "certainly spend their larval life in the sea.")

Bethe, A.

'95. Studien über das centralnervensystem von *Carcinus maenas* nebst angaben über ein neues verfahren der methylenblaufixation. Archiv f. mikroscop. anat., vol. 44, p. 579–622, pl. 34–36.

'97. Das nervensystem von *Carcinus maenas.* Ein anatomisch-physiologischer versuch. Archiv f. mikroscop. anat., vol. 50, p. 460–546, pl. 25–30.

Bordage, E.

'93. Note sur l'étude comparée du système musculaire des Thalassinidés et des Paguriens. Compte rendu sommaire des séances de la soc. philomathique, Paris, no. 10, p. 3–5, text fig. 1–2.

Bouvier, E. L.

'89. Le système nerveux des crustacés Décapodes. Ann. des sci. nat., zool., series 7, vol. 7, p. 73–106, pl. 7.

'90. Sur l'organization de la *Gebia deltura.* Bull. soc. philomathique, Paris, series 8, vol. 2, p. 46.

'90a. Variations progressives de l'appareil circulatoire artériel chez les crustacés Anomoures. Bull. soc. philomathique, Paris, series 8, vol. 2, p. 179–182.

'91. Recherches anatomiques sur le système artériel des crustacés Décapodes. Ann. des sci. nat., zool., series 7, vol. 11, p. 197–282, pl. 8–11.

'91a. Les Glaucothoés sont-elles des larves de Pagures? Ann. des sci. nat., zool., series 7, vol. 12, p. 65–82.

(Anatomy of Glaucothoe and discussion.)

'92. Observations sur les moeurs des Pagures, faites au laboratoire maritime du Saint-Vaast-la-Hogue pendant le mois d'Aout 1891. Bull. soc. philomathique, Paris, series 8, vol. 4, p. 5–9.

Brooks, W. K.

'99. The foundations of zoology. viii + 339 pp.; New York.

Butschinsky, P.

'94. Zur entwicklungsgeschichte von *Gebia littoralis.* Zool. anzeiger, vol. 17, p. 253–256.

Claus, C.

'61. Zur kenntniss der Malacostracenlarven. Würzburger naturwissensch. zeitschr., vol. 2, p. 23–46, pl. 2–3.

(First and later zoea of "Pagurus." Regarded by the author as possibly a Dromia larva.)

'76. Untersuchungen zur erforschung der genealogischen grundlage des crustaceen-systems. viii+123 pp., 25 text figs., 14 pls.; Wien: 4to.

(The previously described larvae were "Pagurus" and now the mysis stage is described. Discussion of the metamorphosis).

'84. Zur kenntniss der kreislaufsorgane der Schizopoden und Decapoden. Arbeiten a. d. zool. inst. d. univ. Wien, vol. 5, p. 271–318, pl. 1–9.

(Figures ventral arteries of two zoea stages, pl. 9, fig. 53–55.)

'85. Neue beiträge zur morphologie der crustaceen. Arbeiten a. d. zool. inst. d. univ. Wien, vol. 6, p. 1–108, 1 text fig., pl. 1–7.

(Contains a figure entitled "Metazoea of Pagurus" but regarded by many as representing a Galathea larva.)

Czerniavsky, V.

'84. Crustacea Decapoda Pontica littoralia; materialia ad zoographiam Ponticam comparatam. 2. Schr. nat. gesellsch. Charkoff, vol. 13, suppl., 268 pp., 7 pls. (Russian.)

(Two zoeae and postzoeal larva of "*Diogenes varians*," *teste* Bouvier, **'91a.**)

Faxon, W.

'82. Selections from embryological monographs. 1.— Crustacea. Mem. mus. comp. zool., vol. 9, no. 1, 14 pls. and explanation.

(Four zoeae, the glaucothoe, and the sixth stage of "Pagurus," probably *Eupagurus longicarpus*; pl. 12, fig. 18–30, pl. 13, fig. 5–6.)

Giard, A.

'86. Sur la castration parasitaire chez l'*Eupagurus bernhardus* (Linné) et chez la *Gebia stellata* (Mont.). Comptes rend. de l'acad. des sci., vol. 104, p. 1113–1115.

Goodsir, H. D. S.

'42. On a new genus and six new species of Crustacea, with observations on the development of the egg and on the metamorphoses of Caligus, Carcinus, and Pagurus. Edinburgh new philosoph. journ., vol. 33, p. 174–192.
(Note on the first zoea of "Pagurus.")

Henderson, J. R.

'88. Report on the Anomura collected by H. M. S. Challenger during the years 1873–76. Report on the scientific results of the voyage of H. M. S. Challenger during the years 1873–76, zoology, vol. 27, xii + 221 pp., 21 pls.
(Description of *Glaucothoe carinata*, p. 83–84, pl. 9, fig. 1–1a.)

Hesse, E.

'76. Description des crustacés rares ou nouveaux des côtes de France. Ann. des sci. nat., zool., series 6, vol. 3, art. 5, 42 pp., pl. 5–6.
(Curious description of the first zoea of "*Pagurus misanthropos.*")

Krieger, K. R.

'80. Ueber das centralnervensystem des flusskrebses. Zeitschr. f. wissensch. zool., vol. 33, p. 527–594, pl. 31–33.

Latreille, P. A.

'30. Cuvier's Le règne animal, distribué d'après son organisation, pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. 2d ed., 5 vols.; Paris.
(Description of Prophylax, vol. 4, p. 78.)

Marchal, P.

'91. Sur un Pagure habitant une coquille sénestre (*Neptunea contraria* Chenu). Bull. soc. zool. de France, vol. 16, p. 267–269.

'92. Recherches anatomiques et physiologiques sur l'appareil excréteur des crustacés Décapodes. Archiv. de zool. expérim. et gén., series 2, vol. 10, p. 57–275, text fig. 1–20, pl. 1–9.

Mayer, P.

'77. Zur entwicklungsgeschichte der Dekapoden. Jenaische zeitschr. f. naturwissensch., vol. 11, p. 188–269, pl. 13–15.
(Embryology of "*Pagurus striatus.*")

Miers, E. J.

'81. Account of the zoological collections made during the survey of H. M. S. "Alert" in the Straits of Magellan and on the coast of Patagonia. Crustacea. Proc. zool. soc. London, 1881, p. 61–79, pl. 7.
(Description of *Glaucothoe rostrata.*)

Milne-Edwards, A., & Bouvier, E. L.

'91. Sur les modifications que subissent les Pagures suivant l'enroulement de la coquille qu'ils habitent. Bull. soc. philomathique, Paris, series 8, vol. 3, p. 151–153.

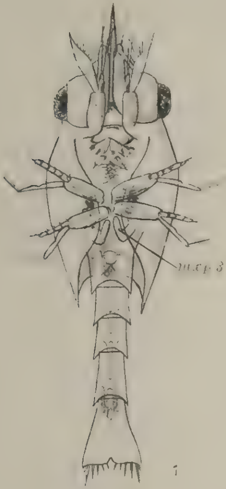
Milne-Edwards, H.

'30. Description des genres Glaucothoe, Sicyonie, Sergeste et Acete, de

PLATE 4.

All the figures are reduced about two thirds in reproduction.

Fig. 1. First zoea, ventral. × 52. *mxp*$_3$, third maxilliped.
Fig. 2. Second zoea, lateral. × 40. *mnd*, mandible; *ul*, upper lip.
Fig. 3. Third zoea, lateral. × 34. *ych*, yellow, *rch*, red chromatophores.
Fig. 4. Fourth zoea, dorsal, swimming position. × 25. *ht*, heart; *ch int*, chitinous gut.
Fig. 5. Glaucothoe or fifth stage, dorsal. × 25.
Fig. 6. Sixth stage, dorsal. × 25. *st*, stomach; *ic*, caecum; *int*, intestine; *ru*, rudimentary pleopod on segment two; *eg*, right liver.



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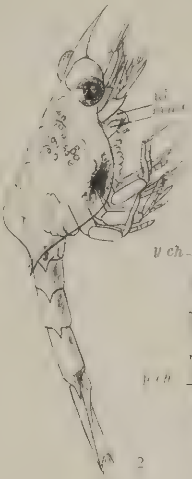

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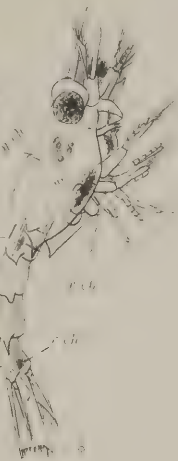

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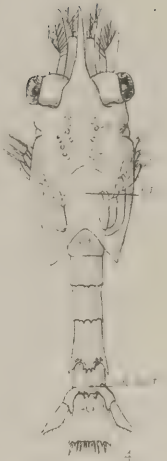

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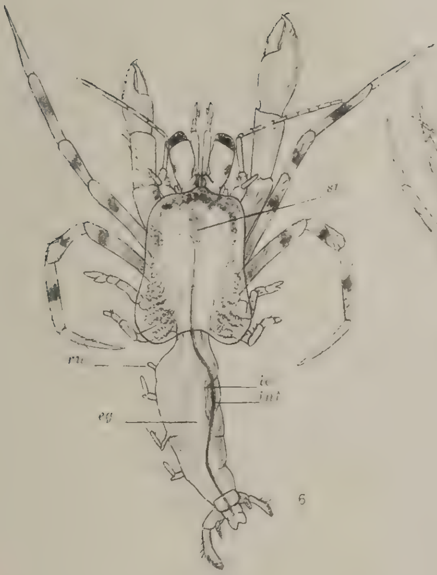

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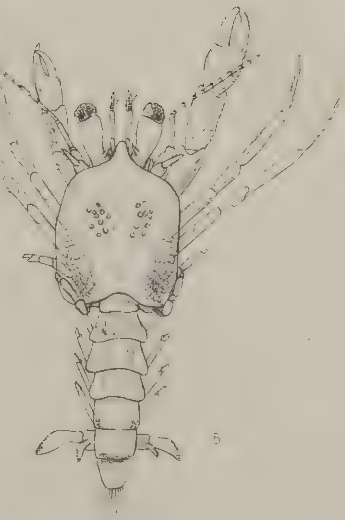

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PLATE 5.

Development of the appendages, showing the structure in the first (1), second (2), third (3), and fourth (4) zoeae, the glaucothoe (5), and the adult (a). For purposes of comparison, the appendages in each series are drawn to a common size.

Abbreviations Common to all Figures.

en — endopod.
ex — exopod.
le — lacinia externa.
li —lacinia interna.
sc — scaphognathite.
prot — protopod.

Fig. 7. First antenna, dorsal.
Fig. 8. Second antenna, dorsal.
Fig. 9. Mandible, ventral.
Fig. 10. First maxilla, ventral.
Fig. 11. Second maxilla, ventral.
Fig. 12. First maxilliped, ventral.
Fig. 13. Second maxilliped, ventral.

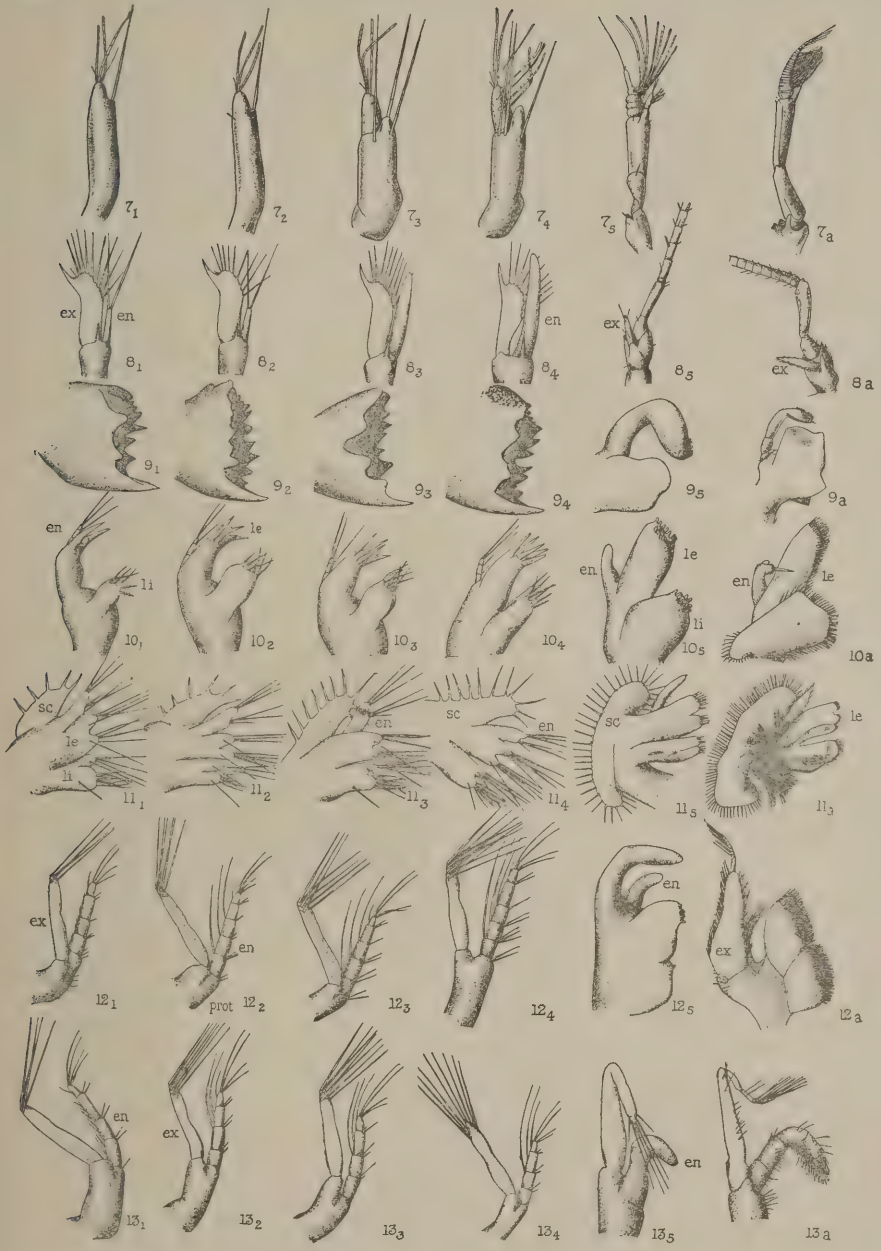

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PLATE 6.

Development of the appendages (continued).

Abbreviations as before ; subscript numbers mark the stages and Roman numerals indicate the thoracic limbs and the abdominal segments.

Fig. 14. Third maxilliped, ventral.
Fig. 15. Thoracic limbs, first zoea, lateral.
Fig. 16. Thoracic limbs, second zoea, lateral.
Fig. 17. Thoracic limbs, third zoea, lateral.
Fig. 18. Thoracic limbs, fourth zoea, lateral.
Fig. 19. Fourth limb, glaucothoe, dorsal.
Fig. 20. Fifth limb, same, dorsal.
Fig. 20a. Tip of same, adult.
Fig. 21. Abdomen of fourth zoea, showing pleopods, ventral.
Fig. 22. Pair of pleopods of fourth zoea more enlarged, lateral.
Fig. 23. Pleopods of glaucothoe, drawn in their respective proportions.
Fig. 24. Pleopods of adult female, drawn in their respective proportions.
Fig. 25. Same of adult male.

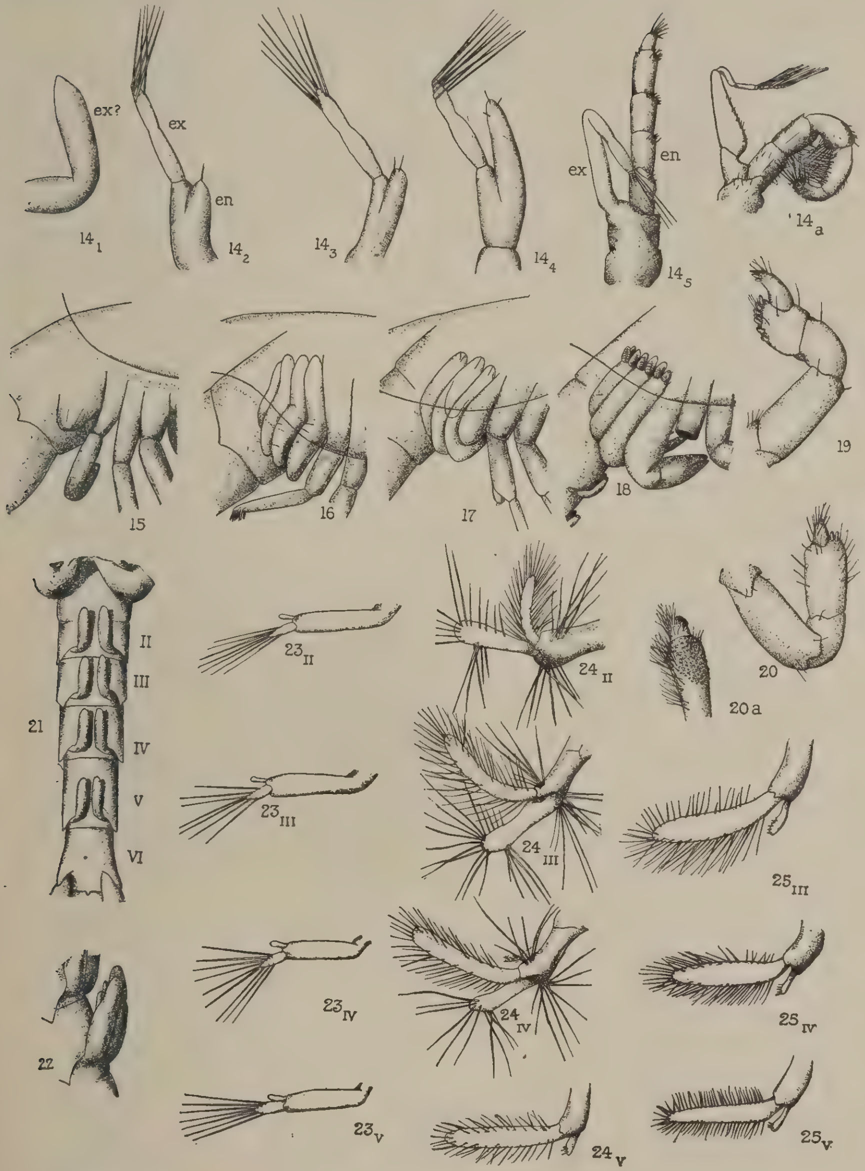

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PLATE 7.

As before Roman numerals indicate thoracic limbs and abdominal segments and subscripts mark the stages.

Fig. 26. Curves showing amount of variability and range of variations in sixth-stage larvae. The ordinates indicate the conditions of rearing; the abcissae the percentages. Continuous line = amount of variability; broken line = range of variations. Conditions are: *nor*, normal; *na*, naked; *sin*, sinistral; *ind*, indifferent. See page 189.

Fig. 27. Part of abdomen, first zoea; living material, showing ganglia and nerves.

Fig. 28. Development of ophthalmic scale. *a*, first antenna; *e*, eye.

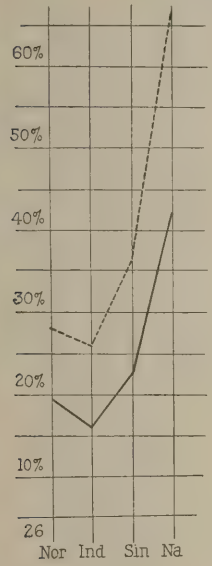
Fig. 29. Ventral surface of infra-oesophageal ganglion, adult. *ar*, ascending arterioles; *comm*, thoracic commissures; *oes*, oesophageal commissures; *st a*, sternal artery.

Fig. 30. *Eupagurus pollicaris*; abdominal ganglia to show arrangement of these parts in the genus Eupagurus. *gn*, ganglionic nerve from fifth ganglion; *comm n*, commissural nerve from fourth commissure; *cp*, columella prominence; *nv*, nerve to fifth thoracic limb.

Fig. 31. Development of telson and uropods; 31_1, 31_2, and 31_4 show the ventral, the others the dorsal surface. Drawn to common size. *ur*, *anlagen* of uropods.

Fig. 32. Curves showing duration of the glaucothoe stage, normal and delayed. Ordinates represent days, abcissae percentages. Continuous line = A, normal; broken line = B1, delay of three days; dotted line = B2, delay of four days.

Fig. 33. Same, naked and sinistral. Continuous line = C, crabs kept without shells; broken line = E, sinistral shells; dotted line = A, plotted with the day and half day percentages added together.



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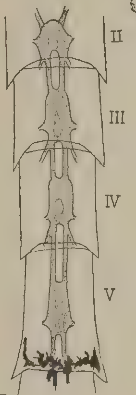

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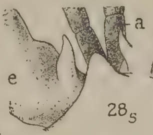
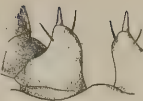
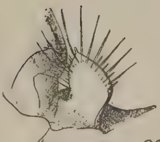


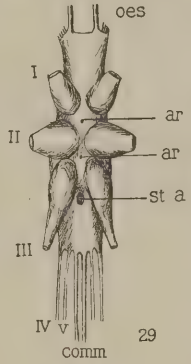
28_5



28_6



28_a



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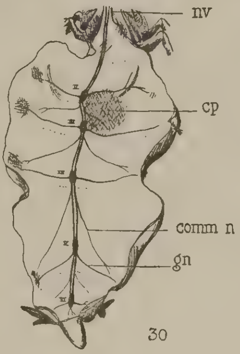

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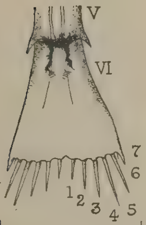

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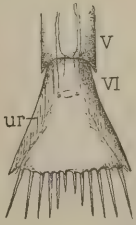
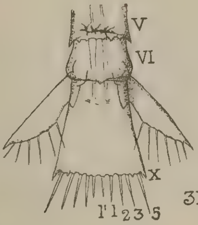


31_2



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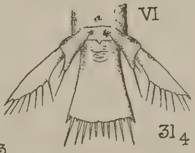
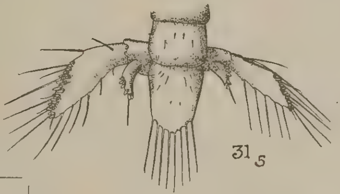
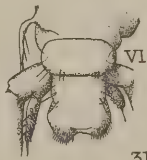


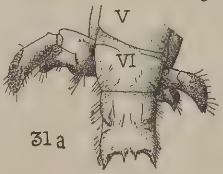
31_4



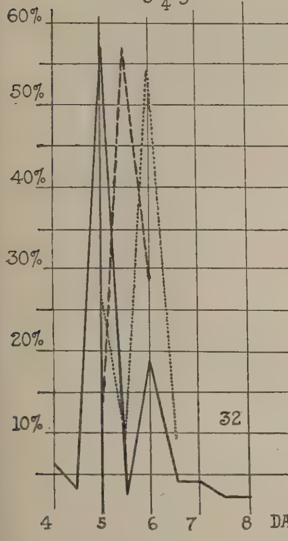
31_5



31_6



31a



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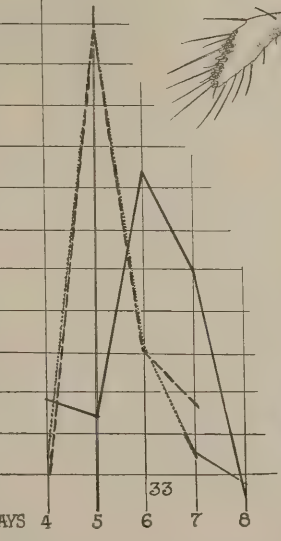

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PLATE 8.

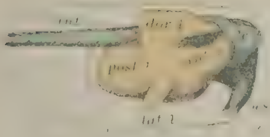
Development of the livers.

All the figures are schematic, but each represents the condition in a single individual, depicted either from a "plotting" on profile paper, or from a wax reconstruction. Allowance must be made for shrinkage and shiftings in the loosely attached organs, especially in figure 34–37, and 44. Figure 34–37 are drawn in relative proportions ; figure 38–42 with a common length of thorax. The slender liver caeca are crowded together in the living animal, but separated here for clearness.

Abbreviations Common to all Figures.

an — anus.
ant l — anterior lobe.
dor l — dorsal lobe.
eg — liver.
g — gonad.
gg — nephrosac.
ic — intestinal caecum.
int — intestine.
lat l — lateral lobe.
lc — lateral caecum.
oes — oesophagus.
post l — posterior lobe.
sd — sexual duct.
st — stomach.
st a — sternal artery.

Fig. 34. Thorax of first zoea, reconstructed ; right, lateral. × 120.
Fig. 35. Thorax of second zoea ; right, lateral. × 200.
Fig. 36. Thorax of third zoea, reconstructed ; right, lateral. × 200.
Fig. 37. Thorax of fourth zoea ; right lateral. × 200.
Fig. 38. Thorax of glaucothoe, example 1, page 165, reconstructed. × 120.
Fig. 39. Thorax of glaucothoe, example 2, page 165, reconstructed. × 160 (?).
Fig. 40. Thorax of glaucothoe, example 5, page 166, reconstructed. × 271 (?).
Fig. 41. Thorax of glaucothoe, example 6, page 166, reconstructed. × 129 (?).
Fig. 42. Sixth stage, reconstructed ; right lateral. × 280 (?).
Fig. 43. Abdomen of mature sixth stage, showing earliest growth of liver caeca ; reconstructed.
Fig. 44. Abdomen of adolescent crab, twenty to thirty days past the glaucothoe ; reconstructed.
Fig. 45. Abdomen of Wareham adult, see page 166. Dorsal ; from photograph of reconstruction.
Fig. 46. Same ; lateral, with intestine partly cut away.



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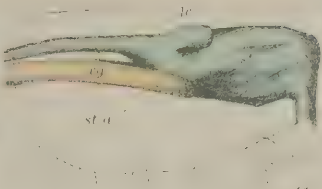

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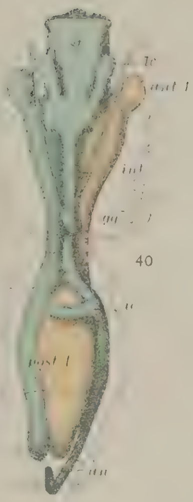

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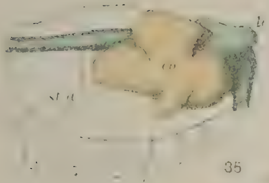

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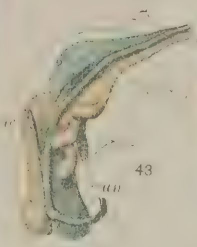

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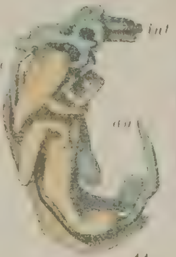

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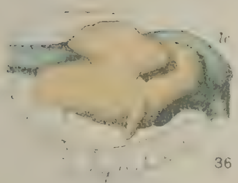

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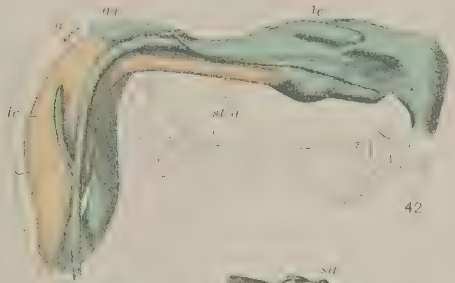

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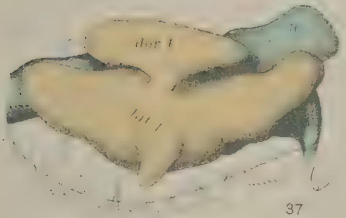

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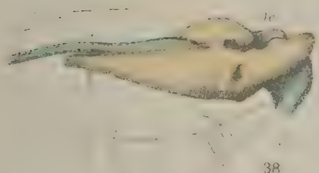

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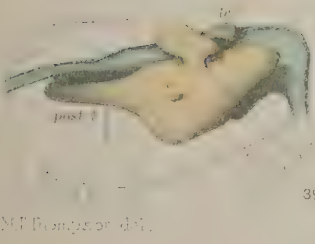

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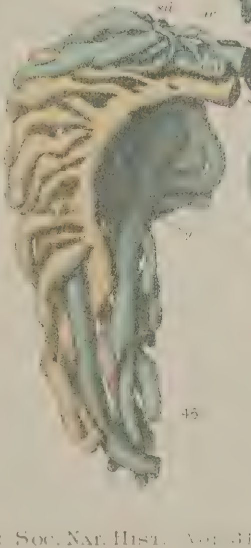

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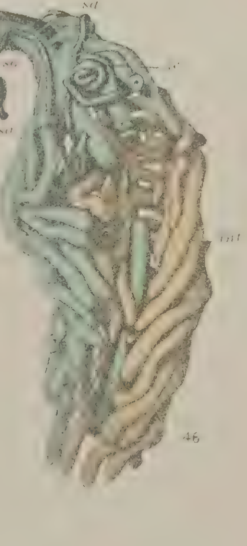

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PLATE 9.

ABBREVIATIONS COMMON TO ALL FIGURES.

a — anterior aorta.
aa — antennary arteries.
ant l — anterior lobe of liver.
car — carapace.
ch int — chitinous gut.
cpv — cardio-pyloric valve.
dlv — dorso-lateral pyloro-intestinal valve.
dor l — dorsal lobe of liver.
dt — dorsal tooth.
dv — dorsal pyloro-intestinal valve.
gg — green gland.
ht — heart.
int — achitinous gut.
lat t — lateral tooth.
lc — lateral caecum.
lpp — lower pyloric pouch.
lv — lateral pyloro-intestinal valve
lvr — lateral-valve ridge.
mpv — median pyloric valve.
mus — muscles passing to limbs.
oes — oesophagus.
oes comm — oesophageal commissures.
op — oesophageal plate.
post l — posterior lobe of liver.
upp — upper pyloric pouch.

Fig. 47–50. Transverse sections, first zoea.

Fig. 47. At beginning of mandibles. × 85. *gang*, posterior cells of supra-oesophageal ganglion ; *st*, stomach.

Fig. 48. Thirty micra farther back, showing approximation of lateral caeca. × 130. *lpp*, site for lower pyloric pouch ; *lt*, lateral tooth.

Fig. 49. Ten micra beyond last. × 85.

Fig. 50. Thirty micra farther back, showing entrance of livers into pylorus, tips of pyloro-intestinal valves and beginnings of floor of intestine, *x*. × 85.

Fig. 51. Transverse section, glaucothoe, showing vertical limb of green gland. × 85. *glob*, globulus ; *ul*, upper lip.

Fig. 52. Transverse section, sixth stage, through the pylorus. × 115. *gang*, ventral thoracic ganglia ; *gg*, canal from green gland to nephrosac ; *upp*, site of future upper pyloric pouch.

Fig. 53. Wareham adult, transverse section of abdomen, showing three liver tubules, the ventral wall of the nephrosac (*gg*), sexual duct (*sd*), chitinous gut, caecum (*ic*), and upper part of flexor muscles (*flex*). × 180.

Fig. 54. Intestine of glaucothoe, longitudinal section through methoria; showing chitinous gut, achitinous gut, and mesodermal sheath of gut, *ms*. × 525.

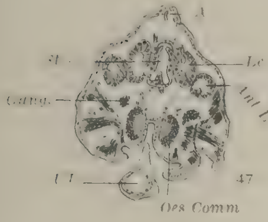
Fig. 55–57. Development of stomach, somewhat diagrammatic, from plottings on profile paper.

Fig. 55. Stomach of fourth zoea, (cardiac part too short). × 184. *lc*, entrance of lateral caecum into pylorus; *eg*, entrance of liver into pylorus.

Fig. 56. Stomach of glaucothoe, (cardiac part too short). × 138.

Fig. 57. Stomach of adult. × 43. *lop*, lower oesophageal plate ; *uop*, upper oesophageal plate.

Fig. 58. Transverse section, glaucothoe, to show union of the pyloro-intestinal valves in the intestine and the partial occlusion of the lumen of the liver. × 180.



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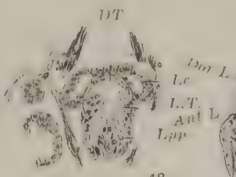

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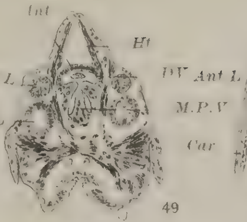

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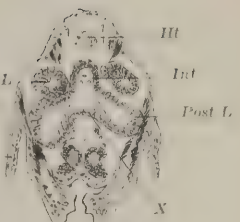

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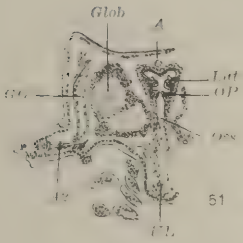

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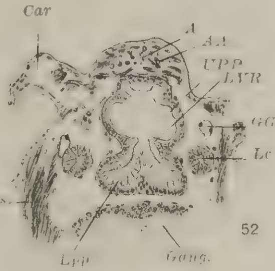

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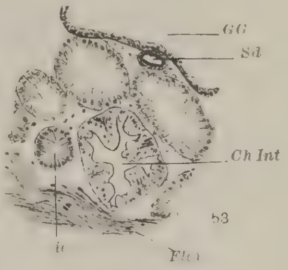

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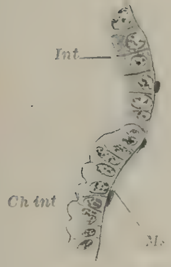

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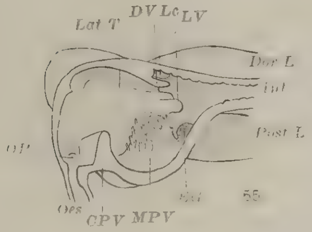

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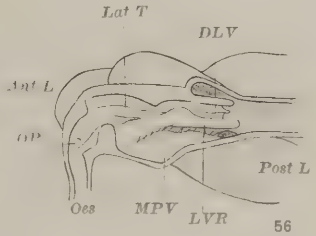

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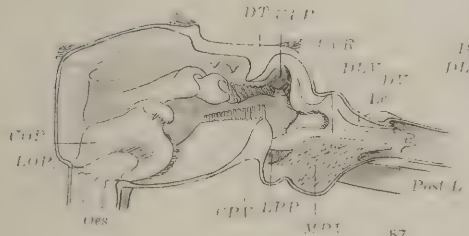

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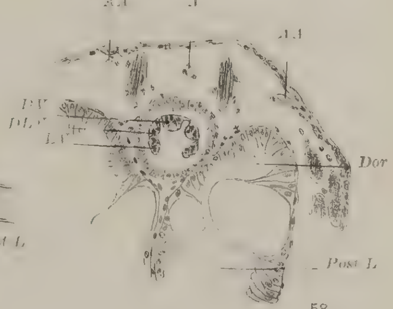

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M.T. [illegible]

PLATE 10.

Roman numerals indicate thoracic limbs and abdominal segments.

ABBREVIATIONS COMMON TO ALL FIGURES.

car — sinus of carapace.
desc m — descending muscle.
en m — enveloping muscles.
ext — extensor muscles.
flex — flexor muscles.
g — gonad.
gang — ganglia.
int — achitinous gut.
lat m — lateralis muscles.
len m — loop-enveloping muscles.
l m — loop muscles.
l seg a — left segmental artery.
nc — nerve cord.
pl m — pleopodal muscles.
post l — posterior lobe of liver.
r seg a — right segmental artery.
st a — sternal artery.
vent — ventralis muscles.
x — descending portion of loop muscles.
x_2 — descending portion of enveloping muscles.

Fig. 59a–f. Muscles of abdomen, glaucothoe, slantingly sagittal; the 1st, 2d, 3d, 4th, 7th, and 10th, of a series of sections each ten micra thick. The transversalis muscles are blackened.

Fig. 60. Diagram of the flexor muscles of glaucothoe; lateralis and ventralis omitted except where they come in contact with the other muscles. Viewed from within.

Fig. 61. Flexors of Cambarus; series of four sections for comparison with sections in figure 59; descending, ventralis, and lateralis omitted for clearness.

Fig. 62. Sexual cells, glaucothoe, reversed by compound microscope. *sa*, posterior aorta. × 370.

Fig. 63. Sexual cells, sixth stage. See plate 8, figure 42. *ms*, nuclei of mesodermal sheath. × 370.

Fig. 64a–d. Development of artery *b′*, as shown in one individual. Example 2, page 165. *sa*, superior abdominal or artery *b*.

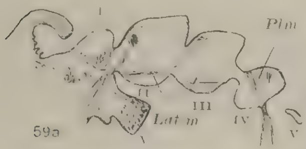
Fig. 64a. Dorsal aspect, plotted from sections. × 220.

Fig. 64b–d. The three successive sections indicated in fig. 64a. × 60.

Fig. 64b. Showing the outer end of the left segmental artery cut longitudinally, and the right artery in cross section.

Fig. 64c. Showing the tips of the segmental arteries passing into the pleopodal muscles on each side.

Fig. 64d. Showing the tips of the segmental arteries in the muscles and artery *b′* running beneath the extensors before it dips toward the flexors.



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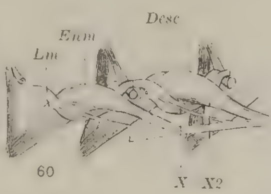

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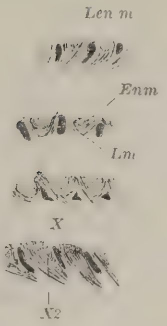

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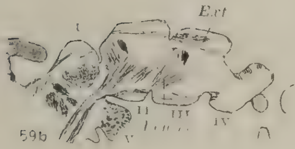

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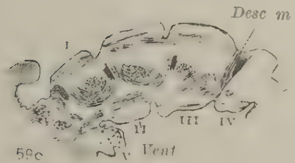

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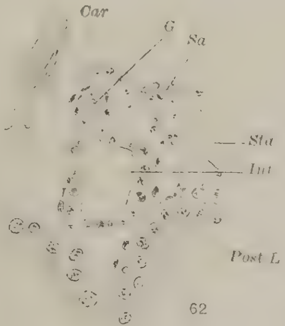

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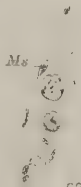

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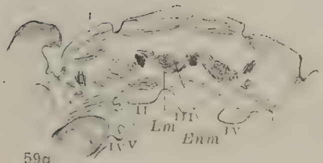

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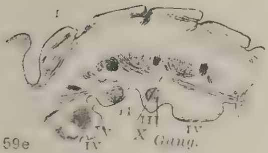

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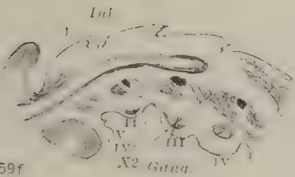

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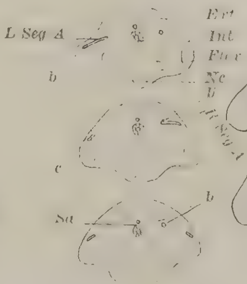

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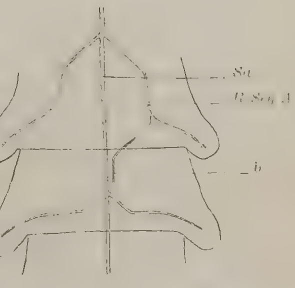

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THE PROBLEM OF LOBSTER CULTURE.

By A. D. MEAD.

Proceedings of the American Fisheries Society, 1905, pp. 156-166.

The Problem of Lobster Culture

BY

A. D. MEAD, Providence, R. I.

THE PROBLEM OF LOBSTER CULTURE.

A. D. MEAD, PROVIDENCE, R. I.

Some years ago the Commissioners of Inland Fisheries of the State of Rhode Island began an investigation of the problems connected with the growth, distribution, and abundance of lobsters, clams, and other shell and food fish of the state. For the last five years particular attention has been paid to the lobster, as the lobster fisheries of the state are exceedingly important and at the same time so extensive as to threaten the total extermination of this delectable food animal.

At the suggestion of Dr. H. C. Bumpus, then one of the members of the Rhode Island Commission, an attempt was made, partly in collaboration of the United States Bureau of Fisheries, to rear lobster fry in sufficient numbers to preserve, or if possible to increase, the supply of lobsters.

For a long time the hatching of lobsters has been carried on artificially by the United States Bureau of Fisheries, but attemps to rear the fry to a size where they can protect themselves and stand some chance of surviving when put overboard. have repeatedly been considered by fish-culturists and biologists, but appeared to present insurmountable difficulties, as all attempts to retain the fry for any length of time in the hatchery proved futile, the mortality being exceedingly rapid.

It hardly seems as if the mere hatching of the eggs would at all increase the number of lobsters, in fact it almost seems as if it would be better to allow them to hatch naturally. The eggs have few enemies, are well protected when attached to the underside of the female lobster, and have every chance of hatching into fry. But the early stages of the fry are unprotected, they swim at the surface and are eagerly sought after by nearly every fish that swims. The real problem of lobster culture is to protect these early fry, to rear them to a stage where they seek the bottom, and hide under stones and weeds, and burrow in the gravel, where they are protected from their enemies and stand a chance to grow into adult lobsters. In this way only can we hope to decrease the usual natural mortality which is estimated amounts to about 999 in every thousand. This was the problem laid out for the Commission by Doctor Bumpus, and this is the problem which has been carried out to a successful solution.

As has already been said the eggs need little or no protection except from man. The mother lobster securely fastens them to the appendages of the under side of her body, carries them safe protected for many months, continually aerates them by the movements of her appendages, and as they slowly hatch, scatters them widespread as she moves about from place to place. Most states recognize the importance of protecting the egg-bearing lobsters. Laws are on the books imposing a penalty for taking, having in one's possession, or selling them, and if a careful inspection by deputies with power of arrest and prosecution is made, a certain protection will be afforded. It is needless to say that at the present time the strict enforcement of these laws is impossible and that many of the short and "egg" lobsters caught, are not returned to the water.

The newly hatched fry, however, are at once the victims of circumstances. They float helplessly about with every shifting current. Everyone who has studied the subject at all has admitted that the early stages of the fry are the critical stages of a lobster's life, and could they be protected and permitted to grow to the stage where they change their habits, seek the bottom and burrow in the sand, the problem of the lobster culture would be solved.

The little lobster which hatches from the egg begins to eat immediately, grows but little until it is about three days old, when it sheds its skin and becomes a considerably larger second stage fry. It remains in this stage on an average four or five days when it moults and grows again and becomes a third stage fry. Again after five or six days it again moults and becomes a fourth stage fry. It is during this stage, that it changes its habits from a free-swimming larva and takes to the bottom to assume the habits of a full grown lobster. The whole process, varying with many factors such as temperature, food, etc., takes from eleven to twenty-one days.

The difficulties connected with rearing the fry to this later

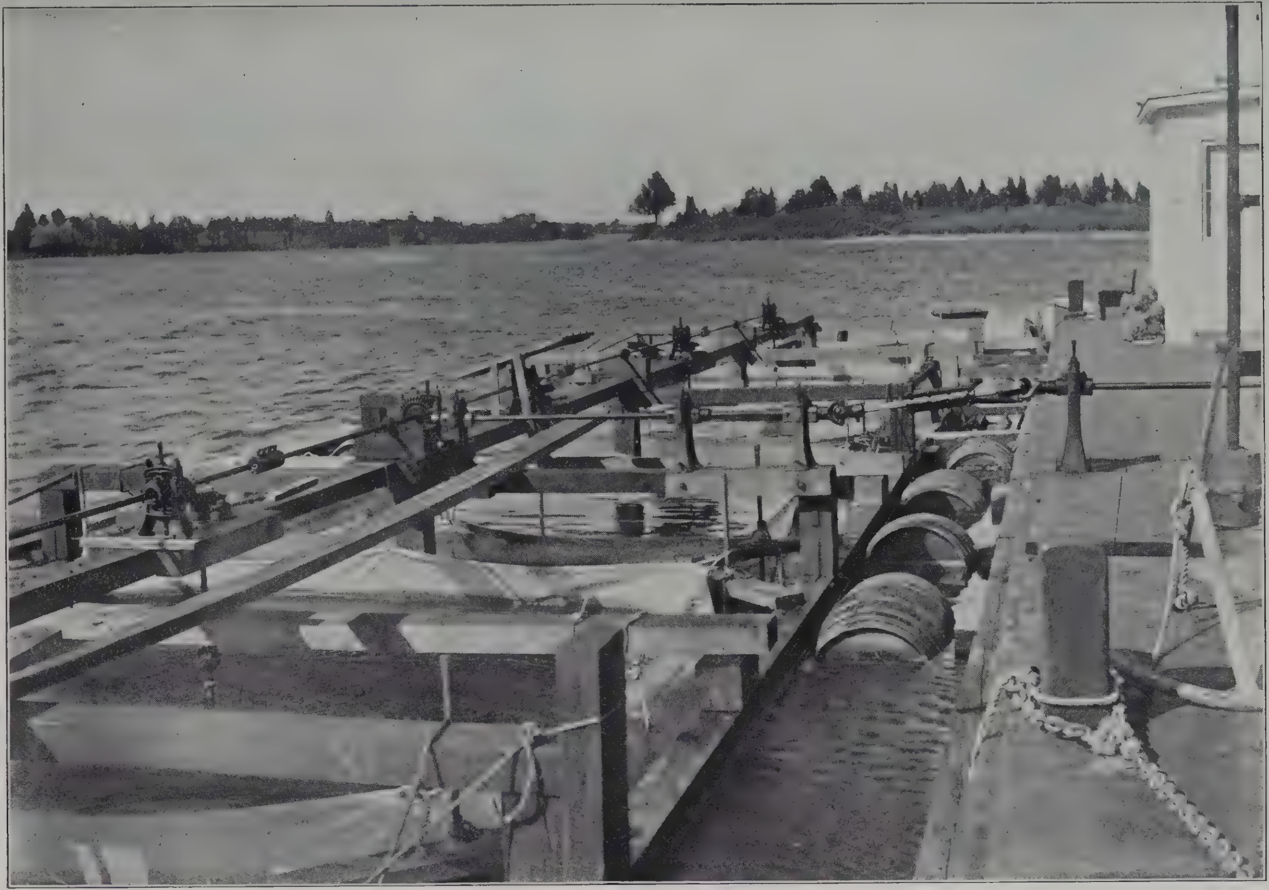

PLATE III.—Showing the appearance of one of the side floats containing the bags for raising the young lobsters. This plate shows, on the right, the universal joint by means of which the power is transmitted to the shafting of the float from the house-boat.

stage are in brief, first, their terrible cannibalism, second, the difficulty of keeping them properly aerated and free from fouling from their decaying food, third, the supply of proper food, fourth, protection from a growth of diatoms and other vegetable and animal forms, which appears on the surface of their bodies and prevents them from swimming and feeding. To combat and overcome these difficulties one by one has been the work of the Rhode Island Commission for the past few years. I will try to review briefly how this has been done.

In 1898 Dr. H. C. Bumpus commenced a series of experiments at Woods Holl, and in 1900 some of them were transferred to the house-boat laboratory of the Rhode Island Fish Commission at Wickford. Up to this time a great many devices for enclosing the fry were tried, and proved to be impracticable, and the one which finally promised the best results was a scrim bag, suspended in the water so that the movements of the tide and wind would frequently change its shape and prevent the fry from collecting too densely in any one place. The meshes of the scrim, of course, allowed a free circulation of water through the bag; but even in this apparatus, when the weather was calm, the lobster fry, together with unused food, would settle into the pockets which were made by the weights necessarily used to keep the bag under water. When the weights were taken off, the least wind would blow the bag out of the water, and this was very trying to the young lobsters. Near the end of the season of 1900 a new principle was applied, on which has depended in a large measure the success of the subsequent work.

After the numerous experiments and watching the results for about five weeks, the conclusion was reached that the secret of success in rearing the young lobsters was to keep the water in continuous motion. This accomplishes two things: it prevents the fry from settling into pockets to smother or devour one another, and it keeps food in suspension, so that the fry can obtain it.

To prove the correctness of this conclusion with the material and apparatus at hand, it was decided to experiment with lobsters which were at that time in small bags. Accordingly the force at the laboratory was divided into watches, and the

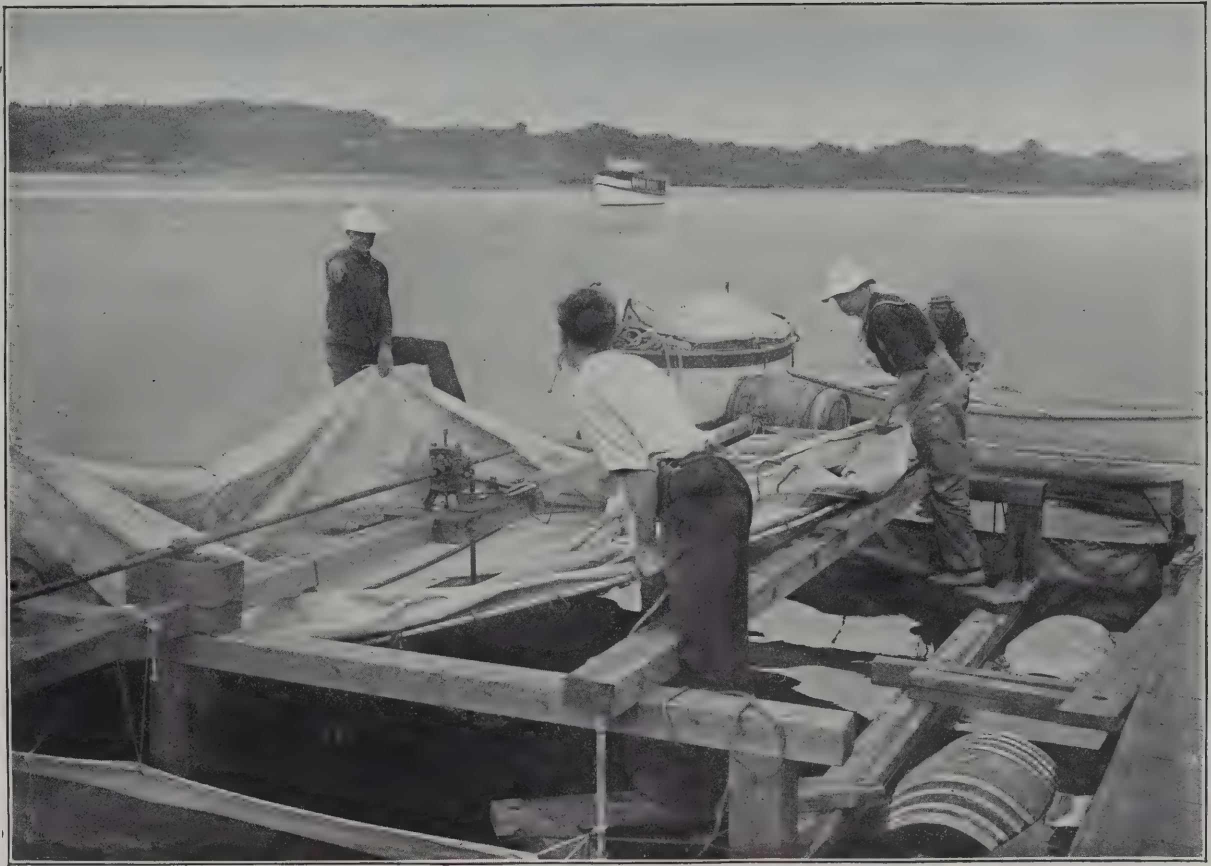

PLATE IV.—Shows the method of "putting down" one of the twelve-foot lobster bags.

water in the bags was thenceforth stirred with an oar continuously for six days. The results was ample proof that the conclusion was correct. There were two lots of lobsters which received this treatment. Neither of these lots was considered to be as promising as the average. However, from one there was obtained 748 lobsters in the fourth stage. This is a larger number than were obtained in any other experiment, either at Wickford, Woods Holl or in any other station where lobster culture has been tried, so far is I am aware. From the other 319 were obtained in the fourth stage; but as one of the bags was old, and had a hole in it, the figures fail to give a correct idea of the results of the experiment. One of the most encouraging results of this method was the clean and healthy appearance of the fry in all stages. The continual stirring prevented the accumulation of parasites found on the body of nearly all of the specimens in the other lots.

During the following seasons this experiment was followed up with others, working upon the same theory, namely; that the water should be constantly stirred. To do this it was necessary to invent a mechanical device which would take the place of the oar and designs for such an apparatus were immediately made.

The mechanical device was put into successful operation in the season of 1901 at Wickford. The apparatus consisted of a number of rotating horizontal paddles, similar to those in use over restaurant tables for circulating air, one in each bag, run by a gasoline engine. The movement of the paddle blades created a constant upward current of water in the bags, which kept the fry off the bottom and kept the food suspended in the water. Through its use 9,000 lobsters were raised to the fourth stage, and in some experiments 50 per cent of the newly hatched fry were carried through to this stage. That this was a decided step in advance of the old methods will readily be admitted by those who have followed the course of previous experiments.

The apparatus now used (1905) comprises a house-boat between the pontoons of which are three small hatching bags 6 x 6 x 4 feet, made of canvas; two side floats constructed of 6 x 6 in. spruce beams bolted together and buoyed by barrels, each supporting five large canvas rearing bags about ten or eleven

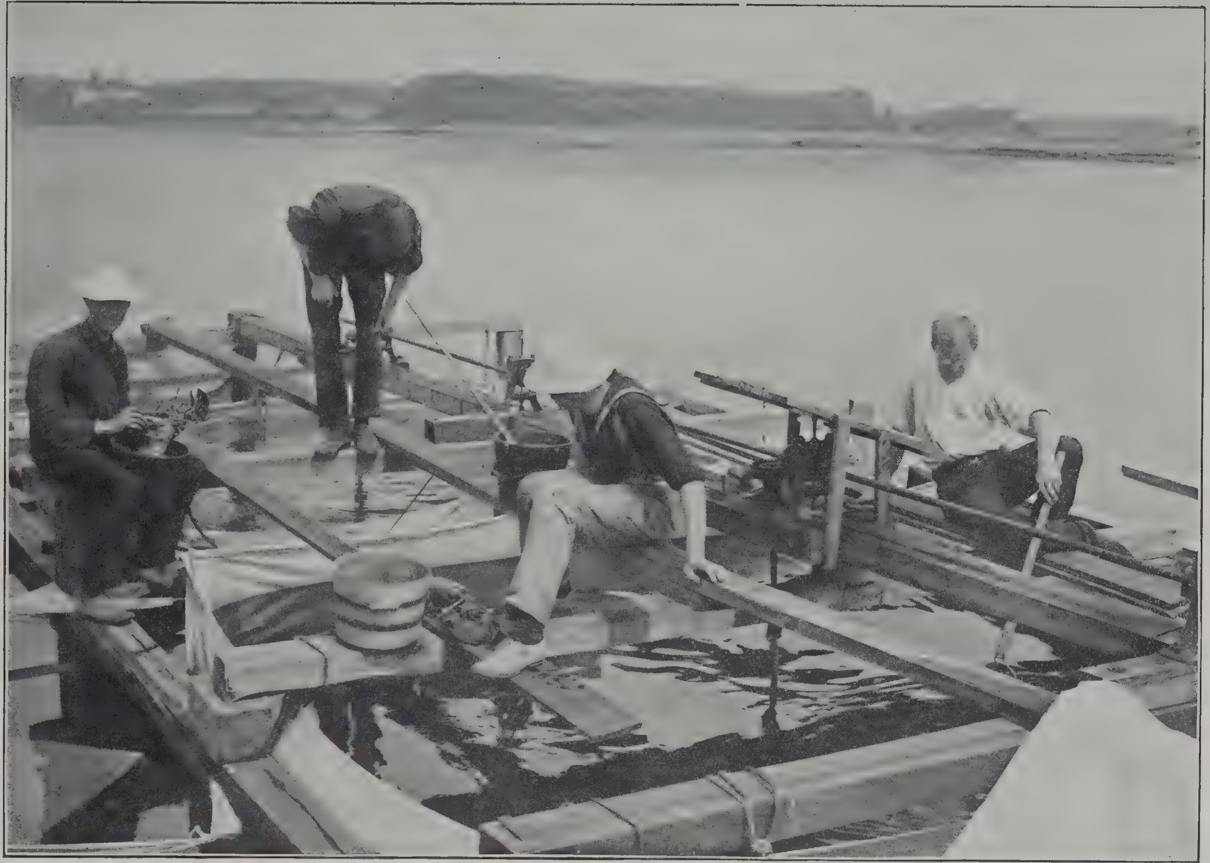

Plate V.—Shows some of the assistants working on a bag. One may be observed counting out fourth stage lobsters, another scraping the eggs from a female lobster preparatory to putting them in the hatching bags.

feet square and four feet deep. Each bag has in it a two-bladed propeller, or "paddle," revolving about ten times per minute, which creates an upward whirling current of water strong enough to keep the fry and particles of food suspended. The vertical shaft of each propeller is geared to one of three longitudinal horizontal shafts; these, in turn, to a transverse shaft which is belted to a two-and-one-half horse power gasoline engine. Each paddle shaft can be thrown out of gear by a lever. The transverse shafts of the somewhat movable floats are coupled to the one running across the house boat by a universal joint and sliding shaft. The latter is a square shaft in two pieces sliding in a sleeve which is cast in two pieces for the sake of economy in manufacture. A drive of 75 feet of shafting is required to reach the farthest paddles, and the bed for the shafts is not, by any means, an example of modern "mill construction." Indeed the floats are constantly bending with the motion of the water, and also warp more or less. The shafts also are almost continually bending, but as they are comparatively light no trouble results from the lack of rigid construction and the transmission is very satisfactory.

The improvements in this phase of lobster culture, namely, that of hatching and rearing to the fourth stage, will, it seems to us, be mainly in the construction of the bags, the feeding of the lobsters, and the prevention of parasitic growth. The latter difficulty, however, is not so serious at Wickford as it was at Wood's Holl. Undoubtedly the percentage of yield can be raised by experimentation along these lines.

It is interesting to trace the actual results of these improvements in apparatus. In the year 1899 in the floating scrim bags at Wood's Holl Doctor Bumpus succeeding in rearing about one hundred lobsterlings to the fourth stage. This was one hundred more than had been reared to that stage previously by any method. In 1900 at Wickford 3,425 fry were reared to the lobsterling stage and 748 of these came from one experiment which was stirred with an oar night and day. This latter number was more than had been reared previously by the combined efforts at all other localities. With the mechanical device of 1901 the number reached 8,974; in 1902, 27,300; in 1903, 13,500; in 1904, 50,597. The total number for the present season, 1905, is 102,000; the entire total for all previous years is 103,796. The largest number reported from any other station is 3,750, reared by an apparatus of the same principle at Wood's Holl in 1902.

In ascertaining the number of lobsters reared to this stage the methods of estimating are not trusted, but the lobsters are counted one by one as they are dipped out of the water with a tea strainer fastened to the end of a stick.

It is obvious, of course, that the output of "lobsterlings" might depend upon the number of newly hatched fry available and on the extent of the apparatus. Taking these things into consideration the comparison is still more favorable to our station, for in most, if not all, other stations the supply of fry has been greater and the per cent of lobsters living through the three moults smaller.

The exact proportions of newly hatched young reared to the fourth stage can be ascertained accurately only by counting the number at the beginning and at the end of the experiment. The time required for counting is so considerable that only in few cases were the fry counted at the beginning of the experiments.

On June 7 and 8, 1905, 20,000 in the first stage were counted and placed in one bag. The "fours" began to appear in ten days and all that lived reached this stage before the end of the twelfth day. 9,635 lobsters in the fourth stage were counted from this twelfth bag; a yield of 48.1 per cent.

On June 28 and 29, 20,000 young fry, of the first stage, were counted into one bag. From this lot 8,178 in the fourth stage were counted; a yield of 40.8 per cent.

These two experiments illustrate very well the general results of the season's work, as there was no extraordinary care given them nor were they conducted under especially favorable conditions.

The proportionate yield is large as compared with that of other stations. The largest of these reported heretofore is 6.6 per cent.; Appelöf, of Norway, and 21 per cent. at Wood's Holl, where the Wickford apparatus was used. The 6.6 per cent was obtained as an experiment beginning with 1,500 fry in the second stage. The 21 per cent was obtained in an experiment beginning with 3,000 (estimated) fry in the first stage.

A higher percentage has been obtained at Wickford with a smaller number; for example, 50 per cent. fourth stage lobsters were obtained from a lot of 1,000 in 1901, but in order to make the results practical they must be conducted on a larger scale.

The question of course arises are the efforts of the Rhode Island Commissioners in thus rearing and planting lobster fry apparent in an increase of lobsters in the waters of the state? The time has hardly come to answer this question. From our experiments it has been determined that it requires some five or six years for a lobster to attain a size of nine inches or more and the planting of fry has not been carried on as yet on a large scale for that length of time. However, in the vicinities where these lobsters were liberated, the lobster fishermen report that, for the last two winters young lobsters of about eight inches in length were abundant along the shore many of them being dug up by the clam diggers and by ourselves, and it is said that small lobsters have not been seen in abundance in these localities for twenty years. The lobster fry planted by this Commission should be about as large as these young lobsters by this time, and it seems probable that this large supply of young lobsters is the result of the efforts of the Commission. Again this past spring the lobster fishermen report a surprising abundance of young lobsters too small to be held in their pots. When the pots were pulled the little fellows scampered out between the slats. It certainly looks as though in a few years more, with continued efforts on the part of the Commission, the lobster industry of the state, now so dangerously near total extinction, might be rescued.

We feel that the efforts of the Rhode Island Commission have meet with success. There are many difficulties to be overcome, and many chances for improvement. Among the subjects to be further investigated is that of the proper food for the fry. We at present feed finely chopped clams. At Wood's Holl finely chopped fish was used. Neither one is a perfect food. With improvement in the food probably a larger percentage could be raised to the fourth stage and in less time.

The presence of a growth of diatoms and other organisms on the fry is another serious difficulty. The amount of this growth varies with different seasons. It can be partially overcome by proper shading of the bags. A warm temperature, clean water, proper aeration and circulation of water, proper food will so hasten development that trouble from this parasite growth will be largely eliminated.

There are many other problems connected with the latter stages of the lobster which are of great importance, both economic and scientific. The Rhode Island Commission has undertaken the solution of some of these. The habits and requirements of the young lobsters which have passed through the earlier stages, the rate of growth and age of lobsters up to the marketable size, the migration of adult lobsters along the shores and to and from waters of adjoining states, their migrations to and from deep water, the rate of growth of large lobsters, the extreme age to which lobsters may live and breed, the rate of moulting of the old and young lobsters, questions connected with the loss of claws or appendages and the relation of the growth of new claws or appendages to growth and moulting.

But these are problems for the future. It is sufficient for us at present that we have worked out a method of lobster rearing which enables us to hatch and rear lobsters on a large scale, is comparatively cheap, at least cheap enough to be practical, is capable of indefinite expansion and which will enable us each summer to rear a large number of lobsters to a stage where they will, when liberated grow into large lobsters in sufficient numbers to restock the waters of the state.

EXPERIMENTS IN LOBSTER CULTURE AT THE WICKFORD STATION OF THE RHODE ISLAND COMMISSION OF INLAND FISHERIES,

1904.

By A. D. MEAD.

Thirty-fifth Annual Report of the Commissioners of Inland Fisheries
of Rhode Island, made to the General Assembly at its
January Session, 1905, p. 33.

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PROVIDENCE, R. I.
E. L. FREEMAN & SONS, STATE PRINTERS.
1905.

VIII. EXPERIMENTS IN LOBSTER CULTURE.

The main effort of the commission in the scientific work was directed last summer to the further perfecting of the methods of lobster culture. Considerable changes were made in the transmission gear of the stirring apparatus used in hatching eggs and rearing the lobsters from the first to the fourth stage. The worm gears were replaced by matched gears, the universal joints were improved, and a new form of sliding shaft invented. Larger gears were substituted for the old ones where the main shafts meet the propeller shafts, adjustable hangers with babbitted bearings were substituted for the small pillow blocks, a device for throwing out of gear each propeller separately was invented and put in operation, and the side floats were extended so as to hold four additional hatching bags. Some changes were made in the manner of inserting the screen windows in the bottom and sides of the bags. All of these alterations turned out to be improvements and worked satisfactorily.

One scheme intended for an improvement turned out to be a lamentable failure. It was a framework of galvanized gas piping put together and set into the bags for the purpose of holding down the bottoms. When the paddles are revolving the upward current of water tends to lift the bottom of the bag in the middle, although it is, of course, held down along the edges. The framework referred to seemed to work admirably for the first two or three weeks. When, just as the first lot of young lobsters was coming into the fourth stage, it was observed that their numbers suddenly diminished with distressing rapidity inside the bags; at the same time many young lobsters were observed swimming around outside the bags. The internal framework had chafed holes in the canvas bags and these had escaped detection, as they were concealed by the framework and were several feet under the surface of the not very clear water. This disheartening discovery was made on the 13th of June, almost exactly in the middle of the hatching season. The accident diminished the count of lobsters reared to the fourth stage by twenty or thirty thousand at a low estimate. The young lobsters, however, were not destroyed, and a large proportion of them had nearly reached the fourth stage when they were thus unintentionally liberated. The bags were immediately taken up, washed and mended, and in a few days were again in operation with a new lot of lobsters newly hatched. The old method of holding the bottom of the bags down, with strips of furring on the underside, was resorted to and the utmost vigilance was thenceforth exercised.

The result of the season's work in rearing lobsters to the fourth stage was far better than ever before, and the output was many times greater than that of any other station as far as we are aware. During the next four weeks about 50,000 young lobsters in the fourth stage were counted out.

In ascertaining the number of lobsters reared to this stage the methods of estimating are not trusted, but the lobsters are counted one by one as they are dipped out of the water with a tea strainer fastened to the end of a stick.

The total number thus counted was 50,597. The largest number reported from any other station is 3,750, reared by an apparatus on the same principle at Wood's Holl in 1902. It is obvious, of course, that the output of "lobsterlings" might depend upon the number of newly hatched fry available and on the extent of the apparatus. Taking these things into consideration the comparison is still more favorable to our station, for in most, if not all, other stations the supply of fry has been greater and the per cent. of lobsters living through the three moults smaller.*

The exact proportions of newly hatched young reared to the fourth stage can be ascertained accurately only by counting the number at the beginning and at the end of the experiment. The time required for counting is so considerable that only in three cases were the fry counted at the beginning of the experiments.

---

*All fry used at Wickford were taken from Rhode Island lobsters, and were hatched in the bags at the house boat.

1. On June 16 and 17, 20,000 in the first stage were counted and placed in one bag. The "fours" began to appear in eleven days and all that lived reached this stage before the end of the sixteenth day. 6,631 lobsters in the fourth stage were counted from this bag; a yield of 33.1 per cent.

2. On June 16 and 17, also, 30,000 were counted into an adjacent bag. These promised an equally good result until near the end of the experiment, when the screen window was accidentally cut with a knife and nearly all the lobsters escaped. The final yield was only 1,446, or 4.8 per cent.

3. On June 29–30 and July 1, 16,599 young fry, most of them in the first stage, were counted into one bag. From this lot 7,343 in the fourth stage were counted; a yield of 44.2 per cent.

These three experiments illustrate very well the general results of the season's work, as there was no extraordinary care given them nor were they conducted under especially favorable conditions.

The proportionate yield is large as compared with that of other stations. The largest of these reported heretofore is 6.6 per cent.; Appelöf, of Norway; and 21 per cent. at Wood's Holl, where the Wickford apparatus was used.*

A higher percentage has been obtained at Wickford with a smaller number; for example, 50 per cent. fourth stage lobsters were obtained from a lot of 1,000 in 1901, but in order to make the results practical they must be conducted on a larger scale.

A sketch of the habits of the young lobster and an account of the several years' experiments undertaken for the purpose of obtaining a practical method of rearing the fry through the early period of life, usually recognized as the critical period in which the greatest mortality occurs, were given in last year's report. Our aim has always been to discover a method which should be effective and to devise an apparatus which should be reasonably cheap and capable of extension to a large scale of operation. These things we have accomplished.

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* The 6.6 per cent. was obtained as an experiment, beginning with 1,500 fry in the second stage. The 21 per cent. was obtained in an experiment beginning with 3,000 (estimated) fry in the first stage.

The apparatus now used comprises a house boat between the pontoons of which are three small hatching bags 6 x 6 x 4 feet, made of canvas; two side floats constructed of 6 x 6 in. spruce beams bolted together and buoyed by barrels, each supporting five large canvas rearing bags about ten or eleven feet square and four feet deep. Each bag has in it a two-bladed propeller, or "paddle," revolving about ten times per minute, which creates an upward whirling current of water strong enough to keep the fry and particles of food suspended. The vertical shaft of each propeller is geared to one of three longitudinal horizontal shafts; these, in turn, to a transverse shaft which is belted to the two-and-one-half horse power gasoline engine. Each paddle shaft can be thrown out of gear by a lever. The transverse shafts of the somewhat movable floats are coupled to the one running across the house boat by a universal joint and sliding shaft. The latter is a square shaft in two pieces sliding in a sleeve which is cast in two pieces for the sake of economy in manufacture. A drive of 75 feet of shafting is required to reach the farthest paddles, and the bed for the shafts is not, by any means, an example of modern "mill construction." Indeed, the floats are constantly bending with the motion of the water, and also warp more or less. The shafts also are almost continually bending, but as they are comparatively light no trouble results from the lack of rigid construction and the transmission is very satisfactory.

The improvements in this phase of lobster culture, namely, that of hatching and rearing to the fourth stage, will, it seems to us, be mainly in the construction of the bags, the feeding of the lobsters, and the prevention of parasitic growth. The latter difficulty, however, is not so serious at Wickford as it was at Wood's Holl. Undoubtedly the percentage of yield can be raised by experimentation along these lines.

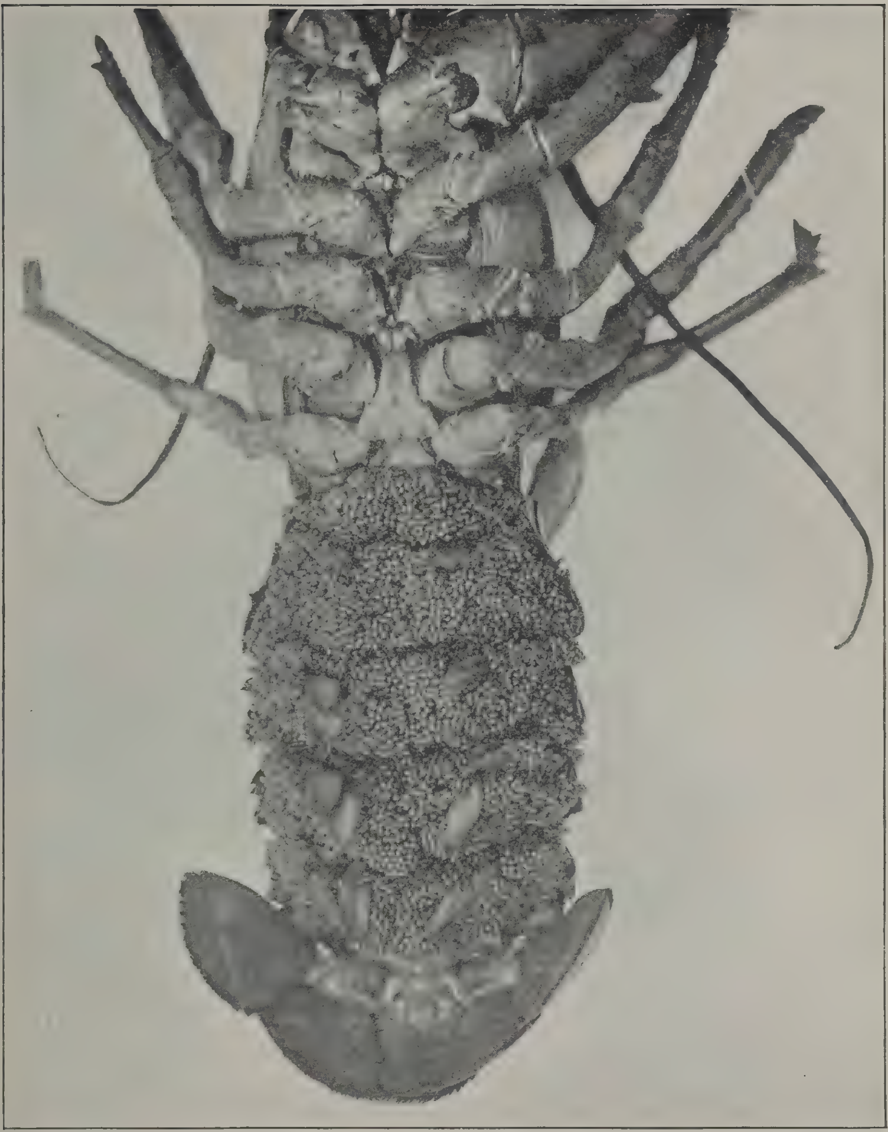

PLATE I.—Shows an adult female lobster in "berry," so called, or bearing the egg-clusters under the tail. (Photograph from life.)

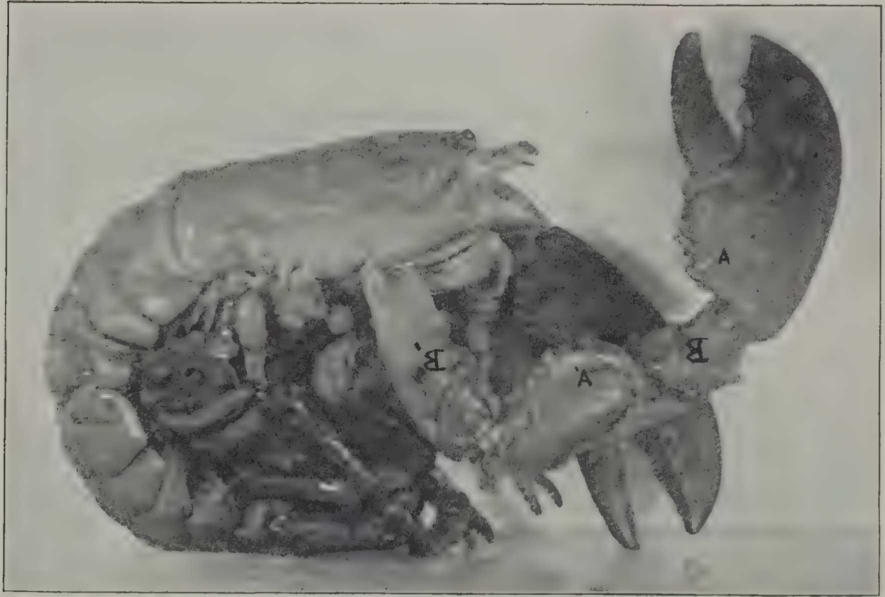

Plate II.—An adult lobster in the process of moulting. It is shown in the act of pulling the largest part of its claw (A′) through the smallest part of the cast-off shell B; A and A′, B and B′, represent relative parts of claw and moult.

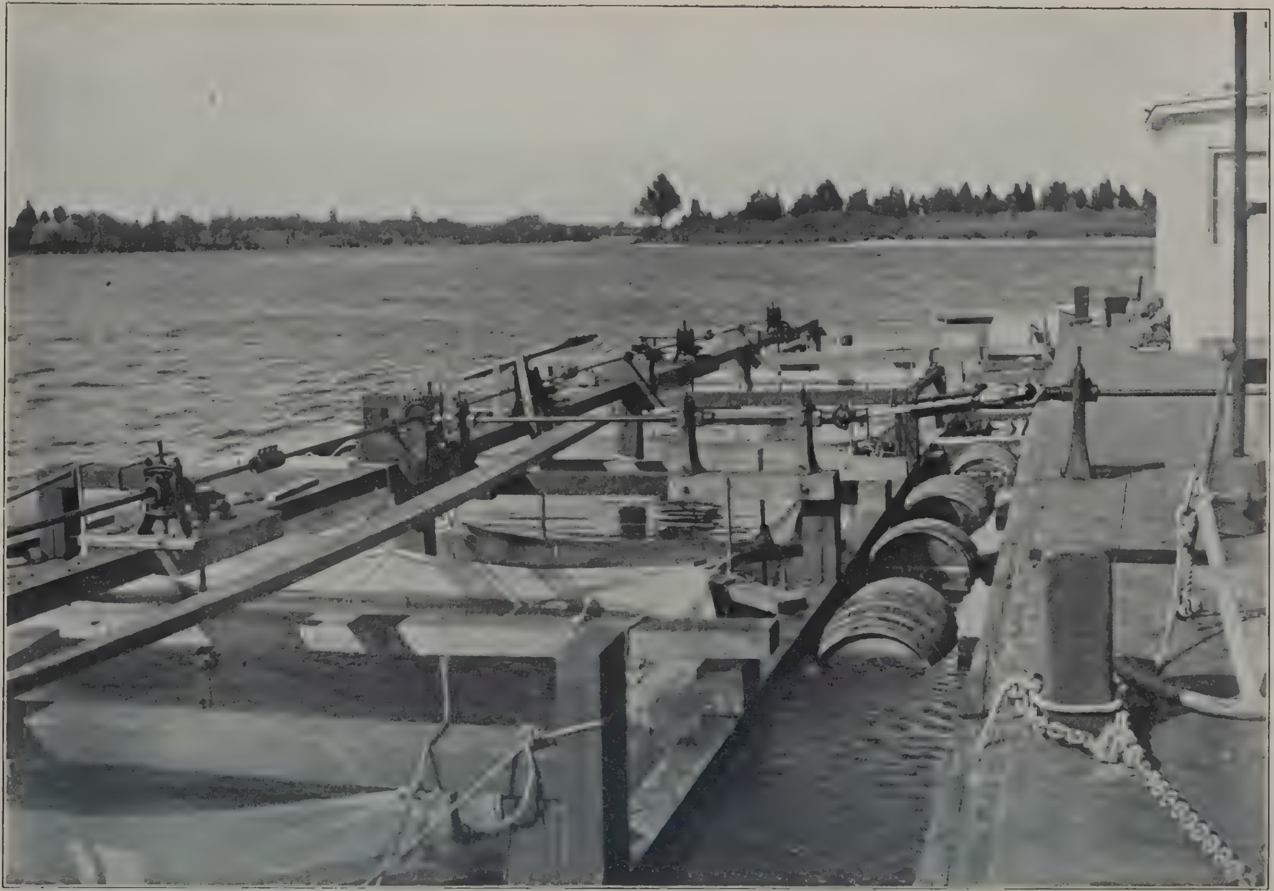

PLATE III.—Showing the appearance of one of the side floats containing the bags for raising the young lobsters. This plate shows, on the right, the universal joint by means of which the power is transmitted to the shafting of the float from the house-boat.

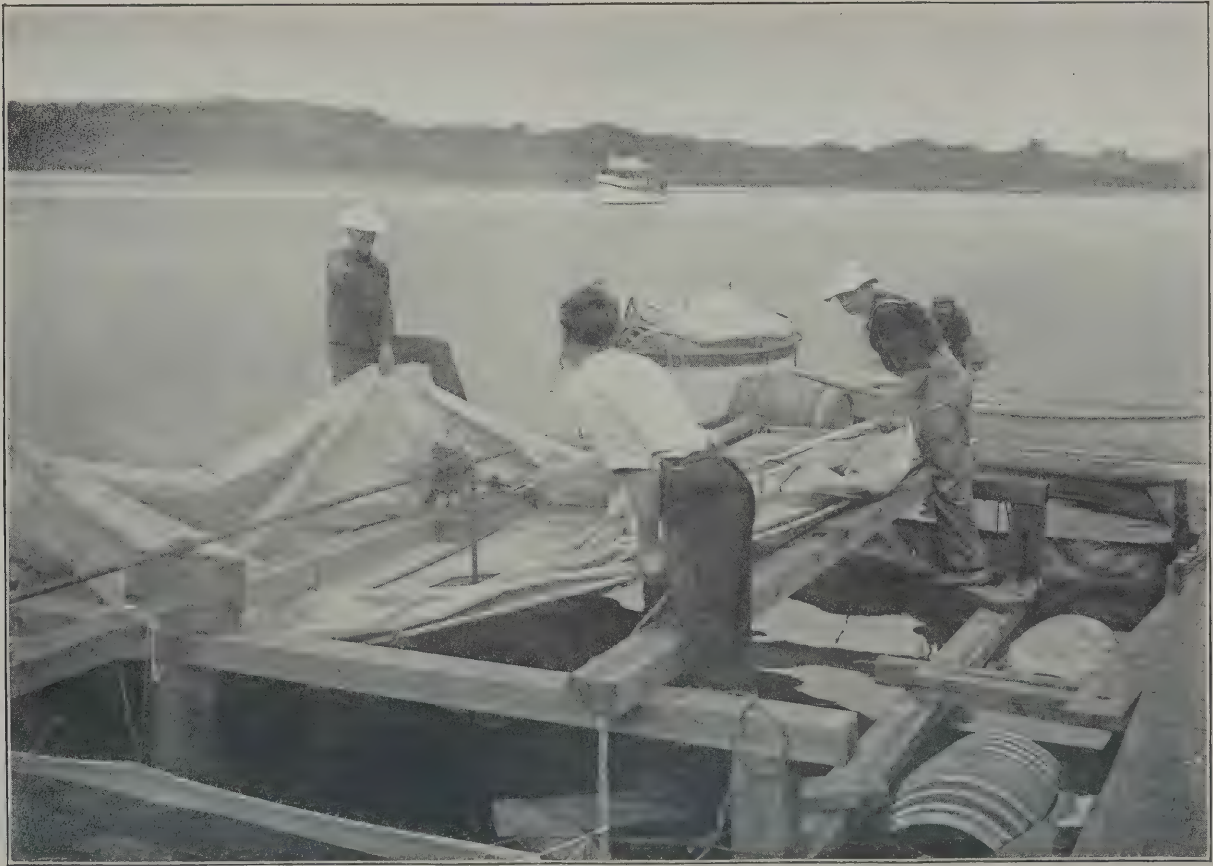

PLATE IV.—Shows the method of "putting down" one of the twelve-foot lobster bags.

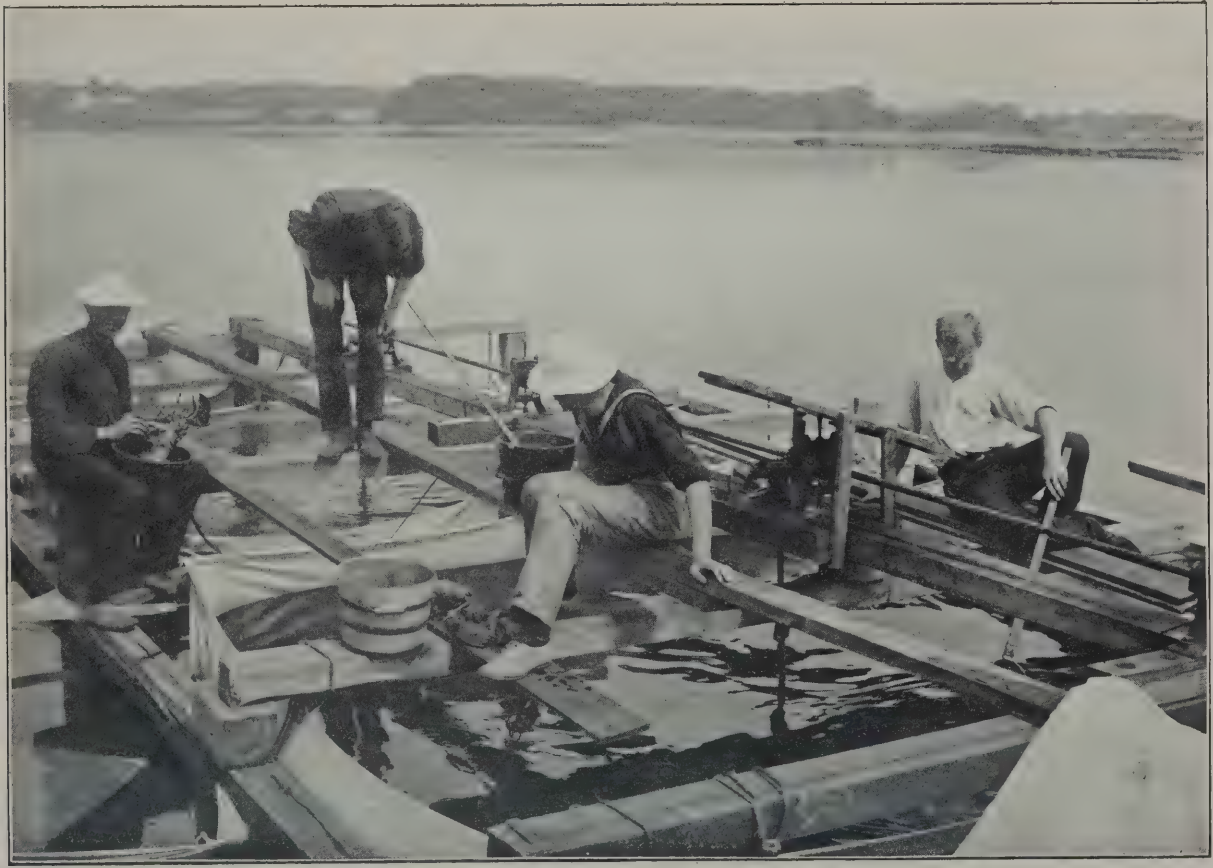

Plate V.—Shows some of the assistants working on a bag. One may be observed counting out fourth stage lobsters, another scraping the eggs from a female lobster preparatory to putting them in the hatching bags.

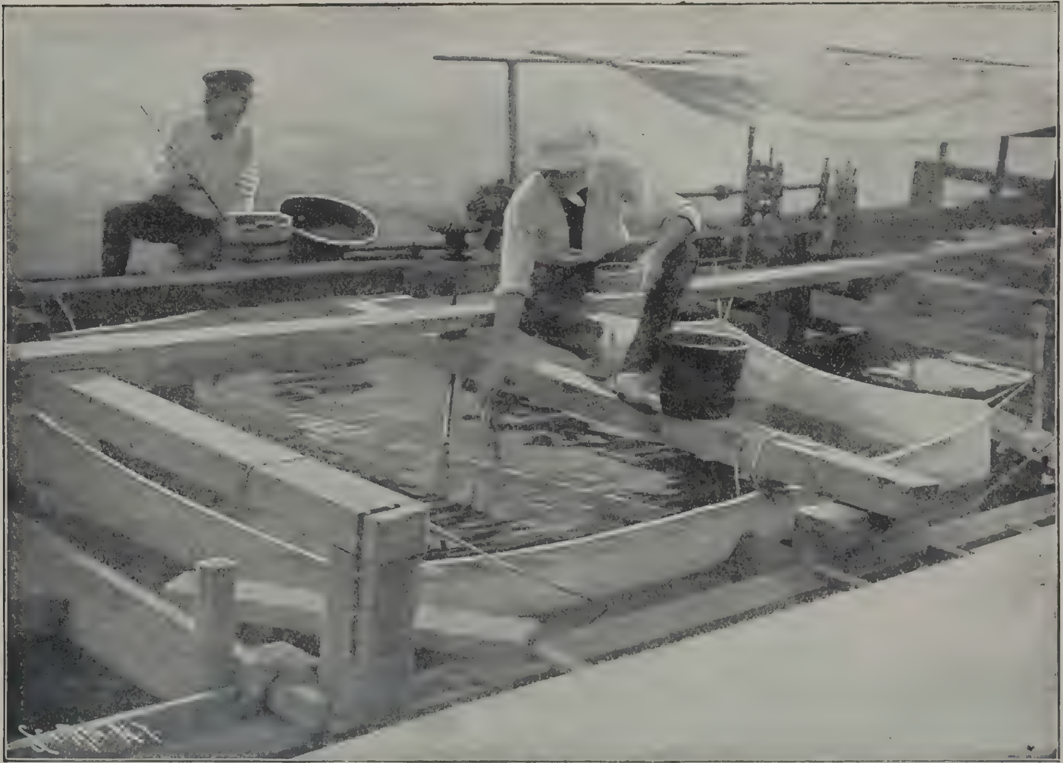

PLATE VI.—One of the large canvas bags in position and showing paddle. Counting out fourth stage lobster. (This picture is published through the courtesy of the "Scientific American."

Experiments with Lobsters in Later Stages.—We have carried out to a practical solution the problem which has always been considered the most difficult and at the same time the crucial problem of lobster culture, the protecting of the young lobsters through the first three moulting periods. Following this naturally comes the problem of protecting the lobsters in the later stages. The successful solution of the first problem has depended mainly upon knowing the habits and requirements of the young fry, and the solution of the next problem must depend upon a similar acquaintance with the habits and requirements of the lobster in the later stages. For several years we have made observations on these later stages and have published some of the results in previous reports.

Even after the lobsters have been reared to the fourth stage their future chances of life depend, in some degree, on the time, place, and manner of their liberation. Thus far it has seemed best to liberate them in the morning, so that they may find hiding places and settle themselves before night falls; to select places along the shore which provide hiding places; and to scatter them over a considerable extent of territory, so they may not gather in a conspicuous swarm.

In order to study the habits and requirements of the lobster at these later stages we have for several years reserved some of those which have reached the fourth stage and placed them in cars. Sand, stones, shells, and sea-weed were put into the cars to give as nearly as possible a natural environment, They seem to thrive in the cars, and a good deal has been learned of their habits. They were fed occasionally on chopped clams, fish, and several other varieties of food. They are always cannibals, but tend to outgrow the bad habit.

Three general facts were brought to light by these experiments: First, at least some of the specimens reared in this way grew as fast as some specimens under natural conditions; for lobsters taken at Wood's Holl in early summer were smaller than some of our specimens a year old. Now those taken at Wood's Holl could not possibly have been of that same season's hatching, and consequently must have been hatched at least a year before. Second, the rate of growth varies enormously even under apparently similar conditions, so that the size of the lobster is no criterion of its age. Third, the young lobsters can withstand the cold of ordinary winters and the freshened water of the spring even in our comparatively shallow estuaries. Some of the specimens were kept over three winters in cars sunk in about 8 to 10 feet of water.

In the experiments just mentioned the rate of growth varied greatly, even among the specimens in the same car. The records of these differences in the rate of growth are published in previous reports, but the following record of the growth of specimens hatched last summer and reared in the cars will serve to illustrate it still further:

Table Showing Growth of Lobsters in Cars at House-Boat During the Summer of 1904.

Car 8. Hatched 6/17–20. Length, Nov. 17.		Car 10. Hatched 6/17–20. Length, Nov. 17.		Car 14. Hatched 6/29–7/1. Length, Nov. 17.		Car 15. Hatched 6/13–7/1. Length, Nov. 17.		Car 16. Hatched 6/13–20. Length, Nov. 17.	
70	$2\frac{3}{4}$	70	$2\frac{3}{4}$	43	$1\frac{11}{16}$	71	$2\frac{13}{16}$	45	$1\frac{3}{4}$
66	$2\frac{9}{16}$	64	$2\frac{1}{2}$	36	$1\frac{3}{8}$	53	$2\frac{1}{16}$	45	$1\frac{3}{4}$
64	$2\frac{1}{2}$	57	$2\frac{1}{4}$	36	$1\frac{3}{8}$	48	$1\frac{7}{8}$	43	$1\frac{11}{16}$
61	$2\frac{7}{16}$	55	$2\frac{3}{16}$	34	$1\frac{5}{16}$	47	$1\frac{7}{8}$	37	$1\frac{7}{16}$
60	$2\frac{3}{8}$	49	$1\frac{15}{16}$	34	$1\frac{5}{16}$	37	$1\frac{7}{16}$	34	$1\frac{5}{16}$
60	$2\frac{3}{8}$	47	$1\frac{7}{8}$	32	$1\frac{1}{4}$	36	$1\frac{3}{8}$	34	$1\frac{5}{16}$
55	$2\frac{3}{16}$	47	$1\frac{7}{8}$	32	$1\frac{1}{4}$	36	$1\frac{3}{8}$	32	$1\frac{1}{4}$
51	2	46	$1\frac{13}{16}$	19	$\frac{3}{4}$	36	$1\frac{3}{8}$	23	$\frac{7}{8}$
51	2	45	$1\frac{3}{4}$			35	$1\frac{3}{8}$		
48	$1\frac{7}{8}$	44	$1\frac{3}{4}$			35	$1\frac{3}{8}$		
48	$1\frac{7}{8}$	44	$1\frac{3}{4}$			34	$1\frac{5}{16}$		
46	$1\frac{3}{16}$	44	$1\frac{3}{4}$			33	$1\frac{5}{16}$		
45	$1\frac{3}{4}$	44	$1\frac{3}{4}$			32	$1\frac{1}{4}$		
45	$1\frac{3}{4}$	43	$1\frac{11}{16}$			30	$1\frac{3}{16}$		
44	$1\frac{3}{4}$	38	$1\frac{1}{2}$			27	$1\frac{1}{16}$		
44	$1\frac{3}{4}$	36	$1\frac{3}{8}$			20	$1\frac{3}{16}$		
43	$1\frac{11}{16}$	36	$1\frac{3}{8}$						
34	$1\frac{5}{16}$	36	$1\frac{3}{8}$						
32	$1\frac{1}{4}$	31	$1\frac{1}{4}$						
32	$1\frac{1}{4}$	30	$1\frac{3}{16}$						
32	$1\frac{1}{4}$								
30	$1\frac{3}{16}$								
30	$1\frac{3}{16}$								
24	$1\frac{5}{16}$								
20	$1\frac{3}{16}$								

Total number, 77.
Extreme length, 71–19 mm. $2\frac{13}{16}$–$\frac{3}{4}$ inches.
Average length, 44.8 mm. $1\frac{3}{4}$ inches.

Observations and Experiments on Individual Specimens in 1904.— In order to study the growth of individual lobsters and possibly to account for discrepancies in rate of growth, color, etc., and in order, furthermore, to study the effect of foods, of loss of claws and regeneration and many similar questions, a large number of fourth and fifth stage lobsters were last summer placed in separate compartments. Each lobster was given a compartment, and a separate record of each lobster was kept until the cars were sunk to the bottom in November.

The lobsters usually selected were in the fourth stage, although it became necessary to take some which had come into the fifth stage. The individual cars were numbered and the lobsters were examined usually two or three times a day in order to keep strict account of the moulting process, color changes, etc. These experiments were attended with some difficulties. At first a high rate of mortality occurred without apparent cause. It was soon discovered that the pine wood, of which the sides of the car were made, caused the death of the lobsters, although the cars were closed at the end with wire netting only and the compartment partitions were of the same material. Cypress or spruce was better, but the lobsters demand a very free circulation of water. This is remarkable in view of the fact that lobsters of this age will sometimes live for weeks in a small dish in which the water has not been changed.

The mortality was especially great at the time of moulting. These are, without doubt, critical periods, and, when a lobster is somewhat weak, are usually fatal. A large number of these specimens died when the skin was partly shed. When all goes well, the moulting is accomplished in a very few minutes, and in a very few hours the skin is destroyed—probably eaten by the former wearer. It is necessary to keep very close watch of the lobster during the fifth, sixth, seventh, and eighth stages, if one wishes to collect the whole skin. In some of the later stages the lobster tries to bury the cast skin. Nor infrequently young lobsters have difficulty in freeing the claws, and sometimes break off the claw autotomously and leave it in the shell. We have several casts which contain a claw broken off in this manner. In a few instances, through a curious deformity of the walking legs, lobsters have tried in vain for days to free themselves from their cast shell.

Color.—Toward the end of the fourth stage light colored spots appear on the lobster, especially on the tips of the claws, back, and end of tail. These never show in the cast shell, but the lobster just emerged into the fifth stage is very brightly marked. The coloration varies in individual lobsters and persists through several stages, but never appears in the casts. The latter are nearly colorless, at least up to the ninth stage, and have usually an even light bluish or purple tint. Mr. Hadley, in a special paper, has dealt with the color changes of the young lobsters more in detail.

Duration of the Interval Between Successive Moults.—One of the prime objects of the experiments in rearing individual lobsters separately was to ascertain the interval between successive moults after the fourth stage. The intervals gradually increase in length up to the fifth stage and that from the fifth to the sixth stage was naturally expected to be still greater. But in our experiments it often was not more than half as long.

The table given below shows a number of cases in which the moulting time was known with accuracy often to the hour of moulting:

DATA ON MOULTING PERIOD OF NORMAL LOBSTERS.

No.	5th Stage. Date.	Size.	Stage Period.	6th Stage. Date.	Size.	Stage Period.	7th Stage. Date.	Size.	Stage Period.	8th Stage. Date.	Size.	Stage Period.	9th Stage. Date.	Size.	Stage Period.	10th Stage. Date.	Size.	Stage Period.	11th Stage. Date.	Size.	Stage Period.	12th Stage. Date.	Size.	Stage Period.
1	July 13..		16	July 29..	21.0	12	Aug. 10..	23.0	14	Aug. 24..	27.0	14	Sept. 10.	33.0		**	40.0		**					
2	Aug. 1	15.0	15	Aug. 1[illegible]																				
3	July 21..		12	Aug. 2..	19.0	14	Aug. 16..	21.0	17	Sept. 20..	24.5													
4	July 23..	17.0	10	Aug. 2..	18.5	14	Aug. 16..	22.0	8	Aug. 24..	26.0	15	Sept. 8.	31.5			34.5							
5	July 23		10	Aug. 2	20.0																			
6	July ..	17.0	14	Aug. 1..	19.5	10	Aug. 11..	23.5	13	Aug. 24..	29.0	15	Sept. 8.	34.5			38.5							
7	July 27..	17.0	19	Aug. 5..	21.0	14	Aug. 19..	25.0		Aug.	28.0													
8	July [illegible]	[illegible]	21	Aug. 7	20.0																			
9	July 28..		18	Aug. 5..	19.0	14	Aug. 19..	23.0	20	Sept. 8..	28.0			33.0			39.0							
10	July 2[illegible]	15.0		Aug.																				
11	Aug. 2	15.0		Aug. ..	18.0	15	Sept. 5																	
12	July 26..	17.0	9	Aug. 4..	20.0		Aug. ..	24.0		Aug. 23..	28.5	18	Sept. 10.	33.0										
13	July 28..	15.0	9	Aug. 6..	18.0	11	Aug. 17..	21.0	14	Aug. 30..	25.5	16	Sept. 15.											
14	July 27	15.0	10	Aug. 6	17.0																			
15	July [illegible]	15.0																						
16	July ..			Aug. 5..	20.0																			
17	July 29..	15.5	6	Aug. 4..	19.0	15	Aug. 19..	23.0	12	Sept. 1..	28.0			32.0										
18	July ..			Aug. 6..	18.0	10	Aug. 16..	22.0	13	Aug. 29..	25.5	19	Sept. 17.											
19	July			Aug. 2	19.[illegible]		Aug.	24.0		Aug. 23	29.5													
20	July	15.0		Aug. 3	18.5	12	Aug. 15.	20.0	17	Sept. 5	22.0													
21	July ..	15.0		Aug. 7..	18.0	14	Aug. 24..	21.0	17	Sept. 10	24.5			29.0										
22	July 25..		8	Aug. 2..	19.0	10	Aug. 12..	24.0	14	Aug. 26..	28.5	19	Sept. 15.	32.5										
23	July ..	16.0		Aug. 4..	19.0	12	Aug. 16..	24.0	11	Aug. 27..	28.5	21	Sept. 18.											
24	July 29	16.0	9	Aug. 7	19.5	13	Aug. 20	22.0	17	Sept. 5.														
25	July 25..	15.5	9	Aug. 3..	19.0	10	Aug. 13..	23.0	14	Aug. 27..	28.0	18	Sept. 14.											
26	July 28	[illegible]	9	Aug. 6	18.5	11	Aug. 17.	22.0	15	Sept. 1														
27	July 29..	13.8	9	Aug. 7..	17.0	10	Aug. 17..	20.0	14	Aug. 30..	23.0													
28	July 27..	16.0	8	Aug. 4..	20.0	13	Aug. 17..	23.0	15	Sept. 1..														
29	July 25..		8	Aug. 22..	21.0	11	Aug. 13..	25.0	14	Aug. 27..	29.0	20	Sept. 16.	34.0										
30	July 26..	16.0	8	Aug. 3..	19.0	11	Aug. 14..	22.0	13	Aug. 27..	25.0	14	Sept. 10.	34.0										
31	July 25		7	Aug. 4.	20.0	11	Aug. 15..																	
32	July 26..	15.5	8	Aug. 3..	18.5	11	Aug. 15..	22.0	12	Aug. 26..	26.0	19	Sept. 14.											
33	July 28	15.0	10	Aug. 7.	18.0	14	Aug. 20.	21.0	13	Sept. 2.														
34	July 28..		7	Aug. 4..	19.5	14	Aug. 18..	24.0	13	Aug. 31..	28.0			31.5			40.0							
35	July 28..	15.0	9	Aug. 6..	18.0	12	Aug. 18..	21.0	12	Aug. 30..	23.0													
36	July 26		10	Aug. 5.	20.0																			
37	Aug. 2	14.5	11	Aug. 13	18.0																			
38	Aug. 1..	15.2	11	Aug. 12..		12	Aug. 24..	21.5	14	Sept. 7..	27.0	12	Sept. 19.											
39	Aug. 2.	1[illegible].2	10	Aug. 12	[illegible]2.0	13	Aug. 15.	25.0	15	Sept. 9														
40	July 30..	16.0	9	Aug. 8.	18.0	12	Aug. 20..	22.0	20	Sept. 9.	26.0													
41	July 2[illegible]	15.0	8	Aug. 6	18.5	12	Aug. 18	22.0	13	Aug. 31														
42	July 27..	15.0	8	Aug. 4..	19.0	10	Aug. 14.	20.0	13	Aug. 27..	24.0	16	Sept. 9.											
43	July 29	15.0	6	Aug. 4..	19.0	10	Aug. 14	23.0																
44	July 29..	14.0	19	Aug. 7..	18.5	13	Aug. 20..	22.0	10	Aug. 30..	27.0	14	Sept. 13.	32.0			38.0							
45	July 29	15.2	8	Aug. 6	18.5	10	Aug. 16.	23.0	14	Aug. 30	26.0													
46	July 29..	15.2	9	Aug. 7..	18.5	13	Aug. 20..	22.0	12	Sept. 1..	28.5			31.5										
47	July 29..	16.5	8	Aug. 6..	20.0	10	Aug. 16..	25.0	13	Aug. 29..	29.5	16	Sept. 14.	36.0			44.0							
48				Aug. 2..	19.0	9	Aug. 11..	24.0	15	Aug. 26..	29.5		Sept. .	33.5			40.0							
49	July 28	16.5	9	Aug. 6..	19.0	12	Aug. 18..	23.0		Sept. ..	30.0			33.0										
50	July 30	15.5	11	Aug. 10..	18.5	12	Aug. 22..	22.0	14	Sept. 5..	26.0			32.0			38.0			43.0				
51	July 2[illegible]	17.0	14	Aug. 9..	2[illegible].0	13	Aug. 22	24.5	15	Sept. 6														
52	July 29	16.0	9	Aug. 7.	19.0																			
53	July 29	17.0	7	Aug. 5	21.0	13	Aug. 18	24.5	19	Sept. 6.														
54	July 29.			Aug. 5..	17.0	11	Aug. 16..	20.0	18	Sept. 3..	30.0			32.0										
55	July 29			Aug. 5..	18.0	13	Aug. 18..	22.0	13	Aug. 30..	27.5	14	Sept. 13.	29.5			33.0			39.0				
56	July 29	14.8	9	Aug. 7..	18.5	14	Aug. 21..	21.0	10	Aug. 30..	24.5	15	Sept. 14.	29.5			31.5							
57	July 29	14.8	9	Aug. 8..	18.0	13	Aug. 21..	22.0	13	Sept. 3..														
58	July 30	15.0	14	Aug. 13..	18.0	9	Aug. 22..	22.0																
59	July 30	16.0	8	Aug. 7	20.0																			
60	July [illegible]	15.0	7	Aug. 6..	19.0	15	Aug. ..	22.5		Aug. 28..	23.5	13	Sept. 10.	29.0			31.5							
61	July 2[illegible]	14.5	8	Aug. [illegible]	[illegible]	9	Aug. [illegible]	[illegible]																
62	July 2[illegible]		8	Aug. 5..	17.0	10	Aug. 15..	20.0	14	Aug. 29..		16	Sept. 14.											
63	July 2[illegible]	13.5	9	Aug. 6..																				
64	July 29		7	Aug. [illegible]	[illegible]	1[illegible]	Aug. 15.	25.0																
65	July [illegible]	14.0	8	Aug. 7	17.0		Aug.	21.0		Aug. 27				[illegible]										
66	July 27		7	Aug. 3..	20.0	10	Aug. 13..	25.0	12	Aug. 25..	32.*	15	Sept. 9.	37.*			47.*							
67	July 27		8	Aug. [illegible]	18.[illegible]	11	Aug. [illegible]	22.[illegible]																
68	July 27	17.0	10	Aug. 6..	21.0	19	Aug. 25..	26.5	14	Sept. 8..	30.0			37.0										
69	July 27		7	Aug. 3..	20.0	10	Aug. 13..	24.0	12	Aug. 25..	30.0													
70	July 27		9	Aug. 5..		14	Aug. 19..																	
71	July 2[illegible]	15.0	9	Aug. 7..	21.8	15	Aug. 22..	21.5																
72	July 27		11	Aug. 7..	19.5	12	Aug. 19..	21.0	15	Sept. 3..														
73	July 27		7	Aug. 3..	19.0	10	Aug. 13..	22.0																
74	July 27		8	Aug. [illegible]	[illegible]	1[illegible]	Aug. 17	[illegible]	17	Sept. [illegible]														
75			8	Aug. 3..	20.5	14	Aug. 17..	25.0	21	Sept. 7..	29.0													
76	July [illegible]		8	[illegible]	[illegible]	2[illegible]	Aug. 2[illegible]	17.0		Sept. 12														
77	July [illegible]		9	Aug. 1	[illegible]	2[illegible]	Aug. 21	22.0	15	Sept. 8	31.0													
78													Aug. 1	[illegible]			38.0			41.0			51.0	
79										Aug. 5..	25.0	13	Aug. 18..	30.0			34.0							
80										Aug. 6..	25.0	13	Aug. 19..	31.0										
81										Aug. 6..	27.5	13	Aug. 19..	31.0	19	Sept. 7..	37.5							
82														30.0		Aug. 7..	36.0							
83														33.0		Aug. 31..	38.9			44.5				
84													Aug. 13..	32.5	34	Sept. 16..	36.5	25	Oct. 11.	44.9				
		mm.	da.		mm.	da.		mm.	da.		mm.	da.		mm.	da.		mm.	da.		mm.	da.		mm.	da.
	Average.	15.4	9.5	Average.	18.6	12.7	Average.	22.5	14.3	Average.	26.5	16.0	Average.	32.1	....	Average.	37.9	....	Average.	42.9	....	Average.	51.0	....

*Represents a specimen whose growth was much greater and more rapid than that of the average lobster. In the successive stages the gains in length are as follows:

5th to 6th stage 25 %.
6th to 7th stage, 28 %.
7th to 8th stage, 15 %.
8th to 9th stage 27 %.

It will also be noted in connection with this specimen that the successive stage periods are much shorter than those of the average.

The normal percentage of gain in length for the successive stages are as follows:

5th to 6th stage, 22 %.
6th to 7th stage, 21 %.
7th to 8th stage 17 %.
8th to 9th stage, 21 %.
9th to 10th stage, 17 %.
10th to 11th stage, 14 %.

Of the 12th stage lobsters, too few were examined to give valuable results by percentages.

**Unfortunately the difficulty in carrying on observations after September 15 prevented dates on the stage period of the 10th and 11th stage lobsters. The same difficulty prevented complete data for the 9th stage.

Data on the Moulting Period of Lobsters having one or more Regenerating Appendages.

No.	4th Stage. Date.	Size.	Stage Period.	5th Stage. Date.	Size.	Stage Period.	6th Stage. Date.	Size.	Stage Period.	7th Stage. Date.	Size.	Stage Period.	8th Stage. Date.	Size.	Stage Period.
1				July 26..		11	Aug. 6..								
2				July 24..		13	Aug. 6..								
3				July 27..		10	Aug. 6..								
4				July 28..		12	Aug. 9..								
5				July 27..		13	Aug. 9..								
6				July 27		13	Aug. 9.								
7				July 27..		13	Aug. 9..		12	Aug. 21..					
8				July 27..		13	Aug. 9..		14	Aug. 23..					
9				July 27..		13	Aug. 9..		14	Aug. 23..					
10				July 24..		16	Aug. 9..		18	Aug. 27..					
11				July 27..		13	Aug. 9..		15	Aug. 24..					
12				July 26..		14	Aug. 9..		28	Sept. 6					
13				July 26..		13	Aug. 9..		28	Sept 6					
14				July 27..		13	Aug. 9..		18	Aug. 27					
15				July 27..		13	Aug. 9..		29	Sept. 7.					
16				July 27..		13	Aug. 7..		13	Aug. 20					
						da.			da.						
				*Average		12	*Average		19						
17	July 12..		16	July 28..											
18	July 12		16	July 28		†12	Aug. 9								
19	July 12		20	Aug. 1											
20	July 12..		15	July 27 ..		†7	Aug. 3.								
21	July 12..		19	July 31..		†9	Aug. 10								
22	July 12..		15	July 27..		†10	Aug. 6..								
23	July 12		19	July 31											
24	July 12..		16	July 28..											
			da.			da.									
	Average		17	†Average		9½									

*In these sixteen lobsters it will be noticed that the moulting period of the 5th and 6th stages was much longer that the normal period. See previous table.

The average duration of the normal 5th stage was 9½ days, and for the 6th stage 12.7 days, but in these regenerating lobsters the average period for the 5th stage was 12 days, and for the 6th stage 19 days.

†Lobsters 18, 20, 21, and 22 had regenerating appendages in the 4th stage, but had attained nearly normal condition during the 5th stage. In this latter stage it will be seen that the moulting period had dropped back to the normal length of 9½ days.

Regeneration.—There are many facts which lead one to suspect that the loss of claws or other appendages, which is very common, and the consequent regeneration of these parts, retards the rate of growth, directly or indirectly, by incapacitating the lobster for food hunting. The whole question of loss of parts, their regeneration, the reasons for the loss, means of prevention, and the effect upon the growth has been given to Mr. Emmel to solve, and his preliminary paper is given in this report.

Liberation of Tagged Lobsters.—In 1902, 1903, and 1904 many of the lobsters from which the eggs had been taken were liberated with a copper tag, bearing a number and the words "Return to Rhode Island Fish Commission," attached to the beak.

The data relative to the movement of the lobsters during 1902–1903 are given in the last report. In 1902, 112 tagged lobsters were liberated and 16 tags recovered. In 1903, 385 were liberated and 30 tags recovered. In 1904, 397 lobsters were liberated and 45 tags recovered. Thirty of these lobsters had traveled ten miles or more before they were captured. One of them, No. 1366, had traveled eleven miles in nine days. Doubtless some tagged lobsters were taken where tags were not returned.

The following table gives the data collected for the season of 1904:

LOBSTERS LIBERATED IN 1904.

Tag No.	Liberated.		Recaptured.		No. of days free.	Distance in miles.
	Locality.	Date.	Locality.	Date.		
1081	Mill Cove..............	May 27..	Poplar Point..............	May 28..	1	1
1095	" "	" 27..	Bill Dyer's Rock..........	June 7..	11	2½
1064	Wickford Harbor........	" 27..	Whale Rock...............	" 15..	19	10½
1083	" "	" 27..	Fire Rock Ledge...........	" 19..	23	13
1085	" "	" 27..	Whale Rock...............	" 15..	19	10½
1098	" "	" 27..	Poplar Point..............	July 1..	35	½
1101	" "	" 27..	Little Tree Point..........	June 3..	7	1
1114	" "	" 27..	Whale Rock...............	July 14..	38	10½
1145	North of North Point.....	June 2..	Bristol Ferry..............	" 9..	37	8
1148	" " " "	" 2..	Bonnet Point..............	June 25..	23	8
1181	" " " "	" 2..	West Conanicut............	" 23..	21	3¼
1247	" " " "	" 10..	Hog Island Light..........	" 27..	25	7½
1277	" " " "	" 10..	Castle Hill................	July 2..	22	9
1288	" " " "	" 10..	Torpedo Station...........	Aug. 1..	52	7
1305	" " " "	" 10..	Whale Rock...............	July 30..	50	10
1311	" " " "	" 10..	" "	" 14..	34	10
1336	" " " "	" 10..	Rose Island................	" 25..	45	6
1344	" " " "	" 10..	Beaver Tail................	" 9..	29	10
1362	" " " "	" 10..	Whale Rock...............	" 14..	34	10
1366	" " " "	" 10..	Fire Rock Ledge...........	June 19..	9	11
1391	" " " "	" 10..	Seal Rock..................	July 10..	30	11½
1414	" " " "	" 10..	Whale Rock...............	Aug. 8..	59	10
1419	" " " "	" 10..	South of Prudence Island...	July 29..	49	3
1432	" " " "	" 10..	Castle Hill................	" 2..	22	9
1464	" " " "	" 28..	Beaver Tail................	" 14..	16	10
1468	" " " "	" 28..	Narragansett Pier..........	" 30..	32	11½
1478	" " " "	" 28..	Beaver Tail................	" 29..	31	10
1486	" " " "	" 28..	Castle Hill................	" 2..	4	9
1488	" " " "	" 28..	Whale Rock...............	" 30..	32	10
1505	" " " "	" 28..	Dutch Island..............	" 13..	15	6½
1515	" " " "	" 28..	Whale Rock...............	" 14..	16	10

Tag No.	LIBERATED.		RECAPTURED.		No. of days free.	Distance in miles.
	Locality.	Date.	Locality.	Date.		
1510	North of North Point.....	June 28..	Narragansett Pier..........	July 30..	32	11½
1521	" " " "	" 28..	Whale Rock..............	" 14..	16	10
1536	" " " "	" 28..	" "	" 14..	16	10
1538	" " " "	" 28..	" "	" 30..	32	10
1542	" " " "	" 28..	" "	" 14..	16	10
1545	" " " "	" 28..	Narragansett Pier..........	" 30..	32	11½
1552	" " " "	" 28..	Whale Rock..............	" 30..	32	10
1556	" " " "	" 28..	Beaver Tail..............	" 29..	31	10
1567	" " " "	" 28..	" "	" 29..	31	10
1568	" " " "	" 28..	" "	" 29..	31	10
1569	" " " "	" 28..	Whale Rock..............	" 30..	32	10
1586	" " " "	" 28..	Beaver Tail..............	" 29..	31	10
1587	" " " "	" 28..	Whale Rock..............	" 14..	16	10
1591	" " " "	" 28..	" "	" 14..	16	10

Number liberated..	397
Number tags returned..	45

PRELIMINARY REPORT ON THE CHANGES IN FORM AND COLOR IN THE SUCCESSIVE STAGES OF THE AMERICAN LOBSTER.

By P. B. HADLEY.

Thirty-fifth Annual Report of the Commissioners of Inland Fisheries
of Rhode Island, made to the General Assembly at its
January Session, 1905, p. 44.

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(WITH DRAWINGS FROM LIFE.)

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PHIL. B. HADLEY,
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E. L. FREEMAN & SONS, STATE PRINTERS.
1905.

CHANGES IN FORM AND COLOR IN SUCCESSIVE STAGES OF THE AMERICAN LOBSTER.

(*Homarus Americanus.*)

WITH DRAWINGS FROM LIFE.

PRELIMINARY REPORT.

PHIL. B. HADLEY,
BROWN UNIVERSITY, PROVIDENCE, R. I.

The present paper is presented with a triple purpose in view: first, to give a brief review of some of the more common facts which have been discovered in regards to the nature and physiology of the pigments of the American lobster and related forms; second, to present the result of a rather brief experimentation upon this subject, carried on at Wickford, R. I.; and third, to record a series of observations upon the form and color changes which take place in the successive stages of Homarus—a series of observations extending over eleven stages of the lobster's existence, and culminating at the time when he has come into his heritage of the adult color, and probably of the adult structural type.

The observations recorded in the following report were carried on during the past year, 1904, at the experiment station of the Rhode Island Commission of Inland Fisheries, at Wickford, R. I., and also in part at the biological laboratory of Brown University. The work at the experiment station was rendered especially favorable and advantageous by the unlimited and unsurpassed opportunities for carrying on observations upon large numbers of young lobsters alive and under practically normal conditions. Although a few of the observations recorded resulted from a study of individual lobsters in successive stages, the greater body of facts was drawn from the study of large numbers of individual lobsters taken at random from among the thousands of their fellows in the large canvas bags, or, in the case of older specimens, from the storage cars. Insomuch as the present methods for hatching and raising allow a considerable range in water depth (from surface to three feet), in light and shade, in temperature and food supply, it is safe to conclude that practically normal life conditions are secured, and that these give rise to normal conditions in the development of the young larvæ. The course of the observations includes the following considerations:

1. What are the morphological changes that take place in the successive stages of the lobster?

2. What is the nature of the pigmentation of the lobster, and what are the color changes in the successive stages?

A few preliminary statements may be found necessary.

The life of the lobster from the time of hatching to the time of death is, it may be said, but a series of "stages" so-called, each one of which represents a period of its life between any two successive moults or castings of its shell. Of these stages the first four are passed through rapidly, the young creature moulting usually four times in the first twenty days of its existence. It is these first few stages, so quickly passed, which include the most important of the changes that the young lobster undergoes, and these are called the larval stages, denoting the successive emergence of one form from another. In each successive emergence the young lobster is larger than before; thus we can say he grows by moulting, but never grows *between* moults. From the fourth stage on, however, each succeeding stage-period is of longer interval (aged lobsters probably not moulting more than once in several years), and the changes which the young adult, as he is now called, undergoes are correspondingly less distinct or significant, being manifested chiefly in the various color changes and in those alterations in the internal morphology which are concerned with the reproductive organs as the young lobster approximates to the adult structural and adult functional type. Of this last problem, however, concerning the internal changes, it is not the purpose of this paper to treat, its scope being limited to a consideration of the changes in the external structure. This may properly include the general body form and the changes which take place in the swimming, ambulatory, or sensatory appendages, showing how they differ from one stage to another.

I. The Changes of Form in the Successive Stages of the Lobster.

The First Larval Stage.

When the egg membrane has burst and the young lobster is liberated, it presents an appearance little resembling the adult. Owing to the coiling of the abdomen and infolding of the appendages while still within the egg, the young lobster emerges with the abdominal portion curved anteriorly around the head, the final segment lying over the rostrum or beak. This is also folded downward and inward, the whole form of the animal thus approaching oval shape. It is but a few moments after the young lobster is freed from its egg-membrane that several changes occur. The abdomen gradually bends away from cephalo-thorax, the tail fan broadens, the antennæ project forward, the exopodites of the thoracic appendages (which heretofore have lain folded over and somewhat between the legs) straighten and become functional, beating the water with short vibratory strokes, and the first stage lobster, now about 8 mm. in length, begins to shift for himself.

During the progress of the first stage the body form undergoes but slight change. There is noticed only the gradual extension of the abdomen and rostrum which, to the end of the stage, forms a decided arc with the dorsal surface of the cephalo-thorax. There is also a somewhat greater projection of the eyes and a widening of the ventral and lateral region of the cephalo-thorax, due probably

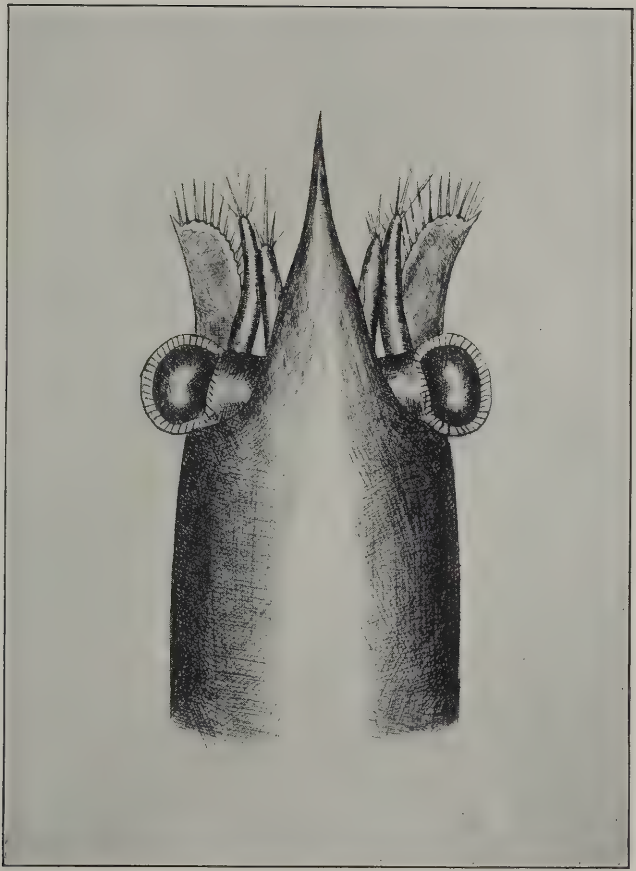

PLATE VII.—Head of lobster in the first larval stage, showing prominence of eyes, and undeveloped state of the first and second pairs of antennæ. (Re-drawn from Herrick.)

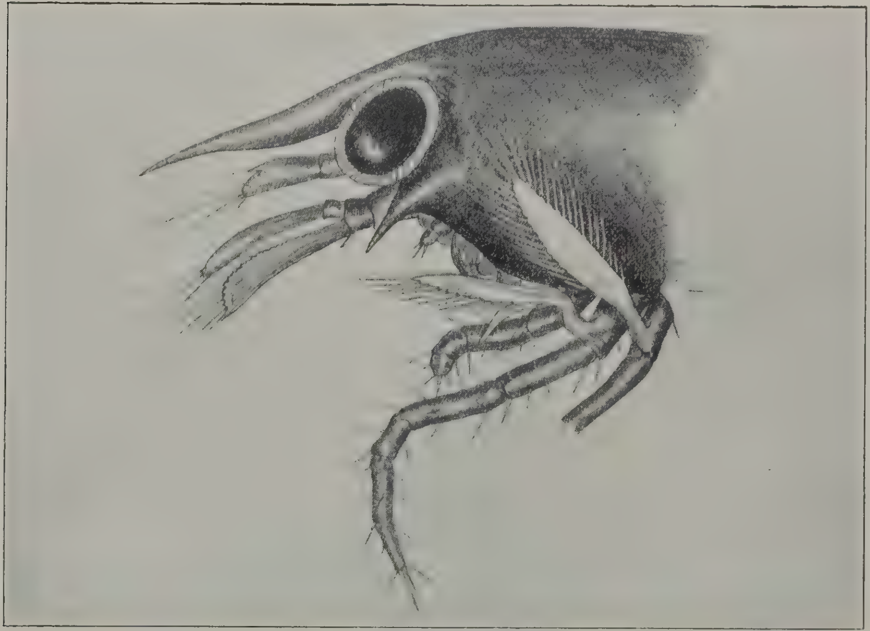

Plate XIII.—Head of lobster in first larval stage, lateral view; shows the budding-off of the inner ramus of the first antennæ from the previously formed external portion of the same. Shows also the swimming appendages (exopodites) and their attachment to the thoracic appendages. (Drawn from life.)

in some degree, to the intense muscular activity of the ambulatory exopodites and to the expansion in the region of the gills as these become functional to a larger degree.

In this first stage the first pair of antennæ project hardly to the end of the rostrum. The endopodites which go to form the smaller and inner members of the first antennæ have just commenced to bud off on the inner side of the outer member, the exopodite, and are each furnished with one long seta. The exopodite has at its end a tuft of four or five smaller setæ. The second antennæ at this stage are composed of two portions: a broad, leaf-like outer portion, the exopodite whose inner margin is curved and supplied with a variable number of feathered setæ, and whose tip holds one sharp spine; secondly, a more slender inner part, the endopodite, bearing setæ at both sides and tip, but whose divisions into antenna segments are not yet visible. (Plates VII, XIII.)

Of the other appendages of the cephalo-thorax, the maxillepeds, the ambulacral appendages, and chelipeds occupy a position much posterior to their relative position in the adult. The second maxillepeds of the first larval stage occupy relatively the position of the chelipeds of the adult, while the chelipeds of the first have their body origin about midway along the ventral border of the carapace. All the walking legs have a correspondingly posterior position relative to that of the adult type. (Plate XVI.)

Of these appendages, the maxillepeds, chelipeds, and ambulatory are all supplied with paddle-like attachments, the exopodites. (Plate XIII). These are furnished with marginal hairs and undergo a rapid vibratory motion downward and somewhat backward. The muscular portion of the basal segments of these exopodites is highly developed, while in the legs themselves, which are not yet functional for crawling, the muscular development is very slight.

The abdomen of the first stage lobster bears no appendages, although the swimmerets, which are to appear in the second stage, can be observed beneath the cuticle in every segment of the abdomen, but the first and last. The last abdominal segment, which now constitutes the "tail-fan," shows no lateral appendages. These do not develop until the third stage.

Second Larval Stage.

When the young lobster has moulted for the first time in its existence, and has entered the second stage, many characterisitc changes form the first larval stage may be observed; it is now somewhat larger, measuring about 9 or 10 mm. (⅖ in.). In the region of the head these differences appertain chiefly to the first and second pairs of antennæ. The inner portions, or the endopodites of the first pair have grown out from the mere buds existing in the previous stage, to half the length of the exopodites or outer portions. These latter, at this period, have developed tufts of setæ which are supposed to have an olfactory function. On both stalks, inner and outer, slight traces of segmentation are now observable. The endopodites are still the larger and thicker of the two branches. (Plates VIII, XIV).

In the second pair of antennæ the endopodites have by this stage grown out to equal the length of the broad, ciliated, leaf-like exopodites, and segmentation is developed to a greater degree than in the previous stage. In the case of the ambulatory appendages, chelipeds, and maxillepeds, there has been a forward shifting of the complete set, so that the body origin of the chelipeds is relatively anterior to the position in the first larval stage and is approximating to the adult position. The other appendages (maxillepeds and walking legs) have shifted to a correspondingly anterior position. The chelæ or large claws, now relatively somewhat larger, have undergone a slight change whereby they more resemble the adult type. No difference between left and right can at this time be determined. The exopodites of all these appendages are still functional to a high degree.

In this second stage the abdomen is characterized by the swimming appendages, or swimmerets, on the under side of the 2nd, 3rd, 4th, and 5th abdominal segments. There is as yet, however, no sign of the appendages modified for reproductive function on the first ab-

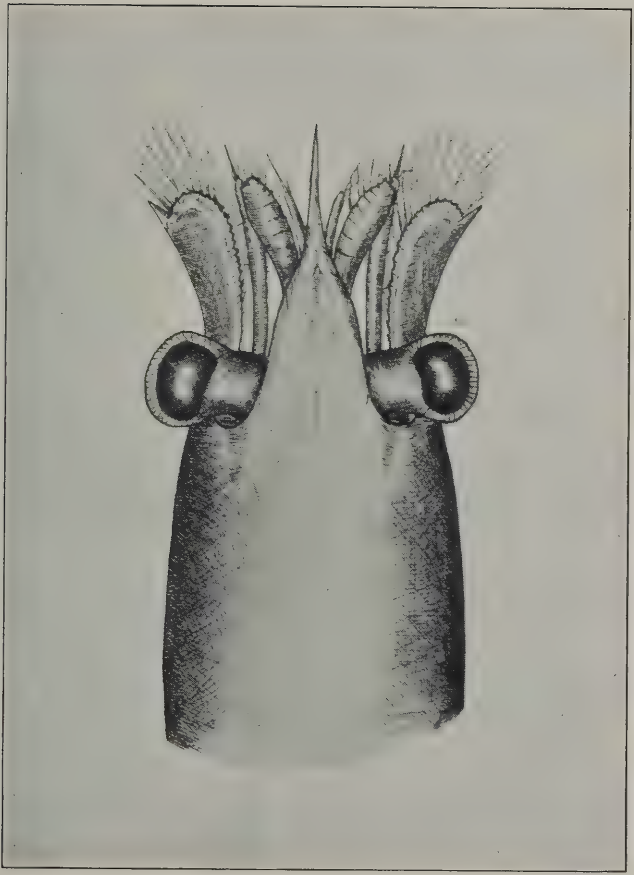

Plate VIII.—Head of lobster in the second larval stage; shows eyes still prominent, and the inner branches (endopodites) which have begun to bud off from the exopodites of the first antennæ. (Drawn from life.)

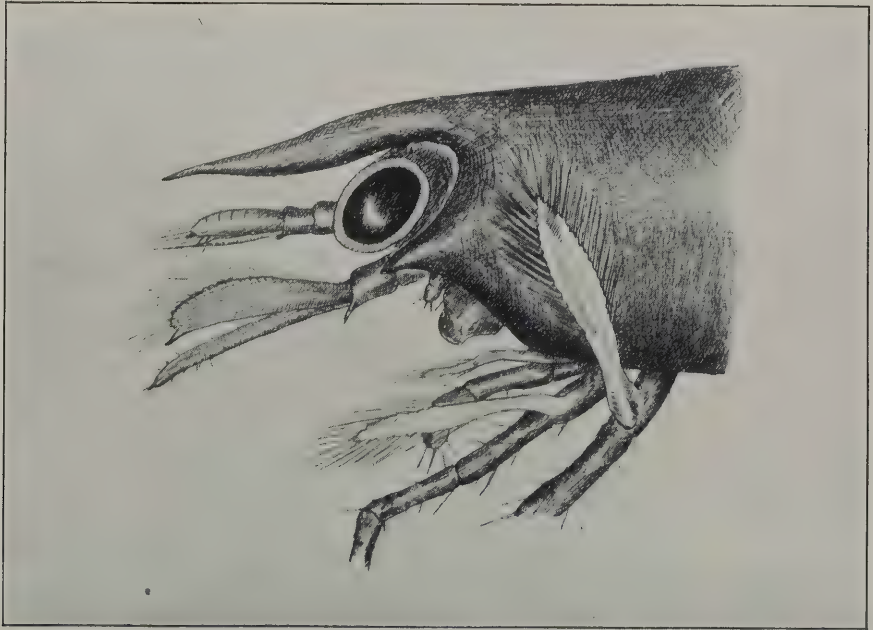

Plate XIV.—Head of lobster in the second larval stage, lateral view; shows the further development of the antennæ. (Drawn from life.)

dominal segment. The appendages of the last may be seen budding beneath the cuticle on the sides of the segment. Thus the tail-fan has not changed from the first stage.

The Third Larval Stage.

The entrance into the third stage is marked not only by an increase in the size of the young lobster, which now measures somewhat over 11mm. (less than half an inch), but in the further change of many of the appendages. The general body form is much the same as that of the two preceding stages, but for the increase in length of the cephalo-thorax (relative to its depth) and the lessening in the convexity of the dorsal surfaces of the same. The eyes have since the first stage been drawn somewhat inward and backward, and are now much smaller compared with the relative size of the body in this stage. We here find also that there may often be one or more post-orbital spines projecting from the carapace in back of the eye, and one spine or more is often found on the dorsal side of the rostrum.

Regarding the antennæ, it is observed that, though the endopodites of the first pair are in diameter much smaller than the exopodites, the former have so increased in length that they project somewhat beyond the latter. The segmentation of both inner and outer portions may be plainly observed at this period. The endopodites of the second pair of antennæ are developed in length somewhat beyond the exopodites, which still retain their broad and leaf-like form and the rim of setæ about the inner margin. (Plates IX, XV.)

The walking legs, maxillepeds, and chelipeds have undergone another forward shifting, so that the position of the chelipeds is still nearer the normal or adult position. These large claws have increased in size so that now in the third stage they are somewhat stronger and larger in proportion to the size of the lobster. There is yet no apparent difference between the left and right claws, a fact which is first observable in a somewhat later stage. (Plate XVII.)

During the earlier part of the third stage the exopodites of the walking legs, chelipeds, and maxillepeds are highly functional.

Towards the end of the stage, however, they seem to function with less ease, and the late third stage larvæ swim about more sluggishly. This is no doubt largely due to the important and marked changes which are taking place within the young lobster as the moulting time draws near and it prepares to enter the fourth stage. It is quite probable that the atrophy which, after the third moult, is noted in the exopodites of the ambulatory appendages and chelipeds, even now in the latter part of the third stage, is retarding the function of these swimming appendages and resulting in the periods of suspended activity which is a phenomenon less often observed in the earlier stages. The following are notes from a record of observations made upon individuals of the third stage to determine whether or not in their activities they showed any signs of adopting in swimming the habits so characteristic of the following stages:

June 11, 1904. About 20 third stage lobsters nearing the time of moulting were placed in a Daniell jar. They swam somewhat less actively than individuals of the second or of the early third stages. Normal attitude in swimming was with head and cephalo-thorax bent downward at an angle of about 45 degrees from a horizontal plane. The abdomen was usually bent downward to a somewhat greater angle. Ofttimes, however, the tail would be straightened out, sometimes slowly with no apparent change in the position of the individual, and again very suddenly, the motion usually resulting in sending the young lobster toward the bottom of the jar. At other times, the tail segments would undergo a rapid contraction, thus sending the young lobster backward with a sudden jerk. This latter action was the most common. The swimmerets were motionless in the process of straight forward swimming, but in the backward jumps, they were more often used together with the tail. The exopodites of the leg sand cheliped seemed functions with greater difficulty than in the earlier stages. It was also noted that very often the exopodites would cease their vibratory motion, and, as if in a period of exhaustion, the young lobsters would sink to the bottom of the jar. Here they would lie for some little time quite motionless

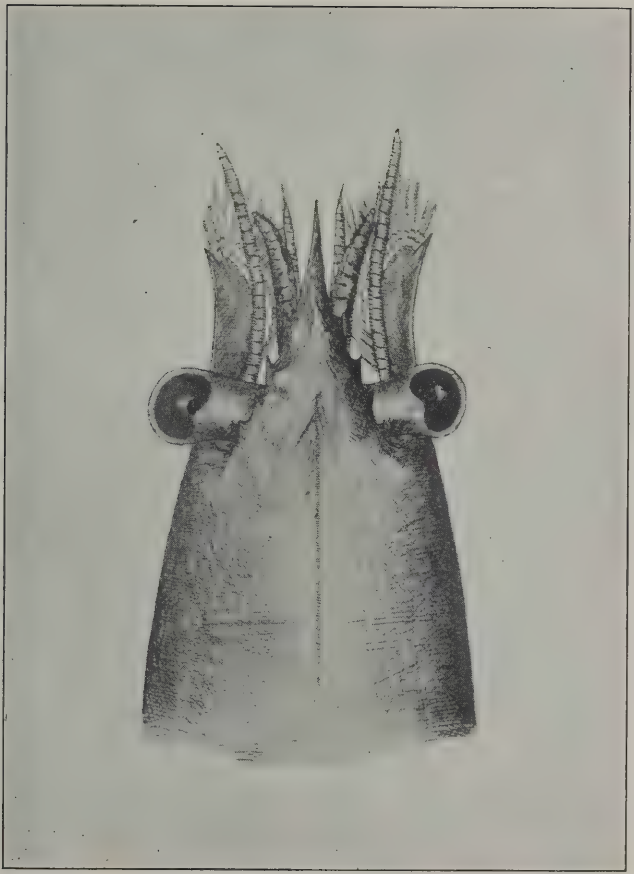

PLATE IX.—Head of lobster in the third larval stage; shows the increase in growth of both second antennæ and endopodites of first antennæ. (Drawn from life.)

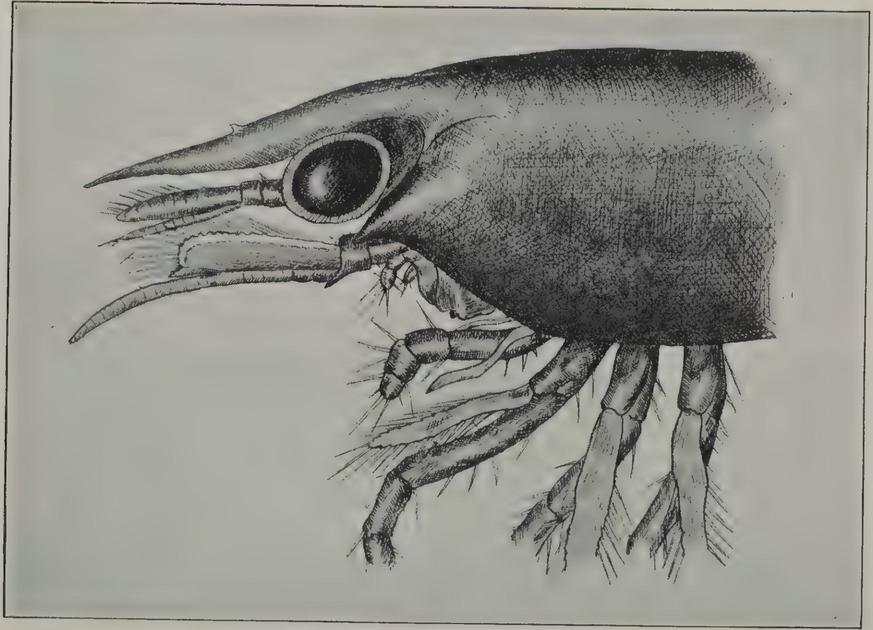

PLATE XV.—Head of lobster in the third larval stage, lateral view; shows the further development of the head appendages, and the position of the swimming appendages of the legs as they appear at the end of the outward stroke. (Drawn from life.)

before again resuming their activities. Although the young lobsters for the most part swim while eating, many times the process is carried on while they are lying on their side or back at the bottom. In the above case, however, there was no food of any kind in the jar and not any trace of masticatory activity evinced. When placed in other jars containing food, the third stage lobsters manifested more perseverance in action than was noted in any preceding stage. Many times an individual lobster would make three or more successive attempts to secure food lost in the first grapple. While swimming near the bottom there were many times apparent efforts to come to rest in an upright position and to support the body by the large claws, but these efforts seldom were successful, and unless active swimming was kept up, an upright position on the bottom could not be maintained.

In the third stage the second, third, fourth, and fifth abdominal segments possess swimmerets differing in no way from those of the previous stage, save that now they are bordered with a delicate fringe of setæ. The last abdominal segment has undergone a decided change since the foregoing stage. A pair of appendages has developed on either side, each consisting of an endopodite and exopodite, respectively. (Plate XVII.) These are bordered with setæ and greatly increase the tail surface upon which depends so greatly the lobster's ability, in later stages, to dart with a single stroke of its tail backward through a remarkable distance. There are still no traces of appendages upon the first abdominal segment.

The Fourth Larval Stage.

When for a third time the young lobster has cast his moult, there emerges an animal which is very different from the third stage and which resembles very nearly the form of the adult lobster. This appearance so suddenly created is due to many changes which will be considered in detail.

In the general body form there has occurred a marked straightening and elongation, the rostrum having developed a number of spines and terminating quite often in a double point (plates X, XX), while the convex curvature of the back has quite disappeared and the tail is much broader. The first pair of antennæ emerge in quite the adult type, of nearly equal length. Each is definitely segmented and bordered at the joints by short setæ. The olfactory setæ are apparently limited to the inner side of the outer branches of the first antennæ.

The second pair of antennæ have undergone a tremendous development since the third stage and now consist of a very long whip-like portion of many segments and the leaf-like exopodite, which has grown but little since the previous stage. The basal joints of this second pair of antennæ seem to have undergone a development so that, by occupying a more anterior position, the antennæ proper are able to fold posteriorily along the side of the body.

The chelipeds of the fourth stage larva are much larger and stronger than those of the third stage, and whereas the latter hung down from the body and were but slightly functional, the former are carried, while swimming, extended straight before the head, and in case the young lobster is disturbed are quickly raised in an attitude of defence. There is as yet no difference between the claws of either side of the body. Both, however, with the passing of the third stage, come to the normal adult position, having their body origin at the anterior end of the cephalo-thorax rather than in a position somewhat posterior, characteristic of the first three stages. (Plate XVIII.) One characteristic feature of this stage is the fact that the chelipeds and ambulatory appendages have lost in the recent moult, the swimming branches, or exopodites. These may be still seen, however, as functionless rudiments, mere stumps with no trace of setæ, on the ischium or third segment of the chelipeds and walking legs. (Plate XXI.) The latter have become far stronger than in the previous stage and readily support the young lobster when he seeks, as he now often does, to crawl along the bottom or hide by burrowing in the sand or under rocks. The anterior pair of walking legs are modified as claws which serve for the purpose of holding prey or to assist in crawling in

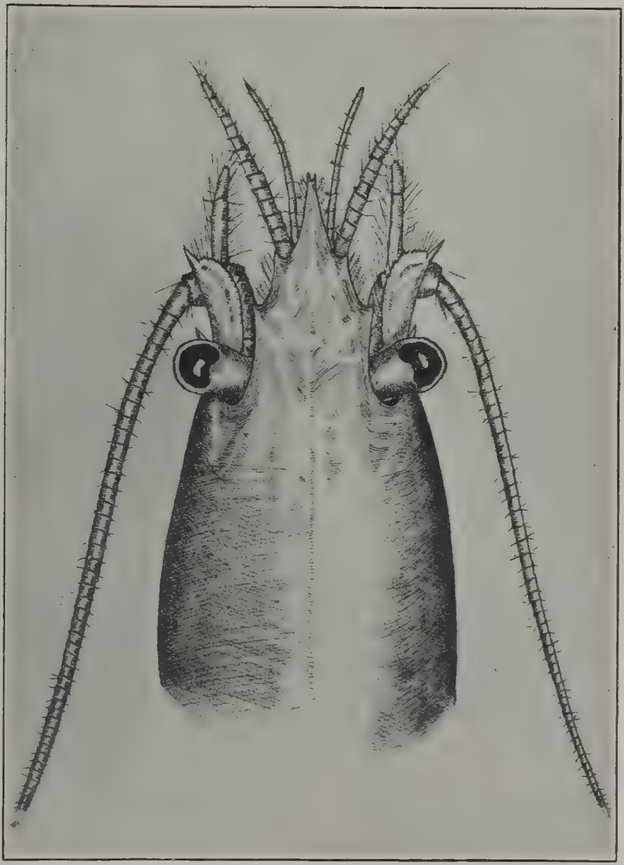

Plate X.—Head of lobster in the fourth larval stage; shows radical change in general appearance. Both branches of the first antennæ have further developed, and the second antennæ have grown out into long slender filaments. The tips of the extended maxillepeds are also evident. (Drawn from life.)

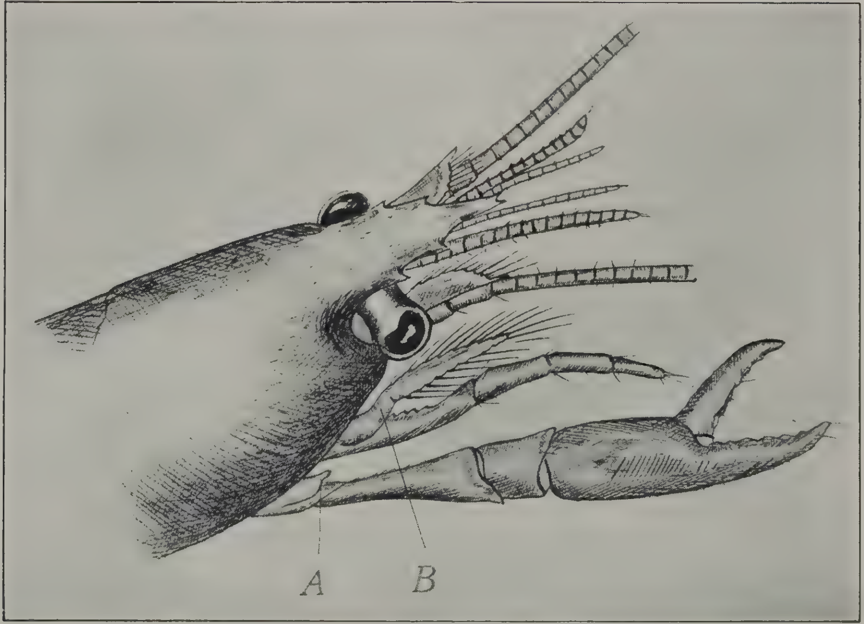

PLATE XVI.—Three-quarters view of the head region of a fourth stage lobster, showing (A) the appearance of the atrophied swimming appendage, or exopodite, of the right cheliped; also (B) the exopodite which still remains attached to the third maxilleped. (Drawn from life.)

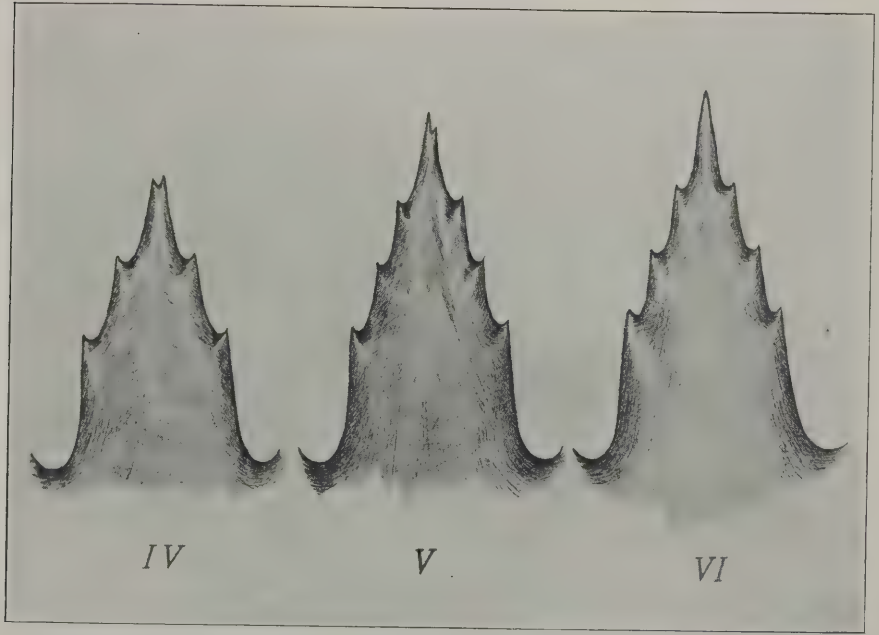

Plate XX.—The rostra of the fourth, fifth, and sixth stages, showing the difference which is commonly observed in the form of the rostrum tip. (Drawn from life)

weeds or grass. They may also be used with the chelæ as weapons of defence. The posterior pair are modified with spike ends, so to speak, and facilitate in crawling over the sand or rocks.

The swimmerets of the second, third, fourth, and fifth abdominal segments have changed from those of the preceding stage. They are now larger and double bladed (having both exopodite and endopodite), and serve to propel the young lobster rapidly through the water or assist in causing, with the agency of the tail, those backward or forward leaps and darts which are most characteristic of the fourth stage. The exopodites of the last abdominal segment have developed greatly since the third stage, and now in length equal the telson itself. The whole tail-fan with all its portions is now fringed with long, closely placed setæ and resembles very closely the tail-fan of the adult type.

After the young lobster enters the fifth stage there are observed few changes as it passes on through the succeeding stages. The general body form of this stage is quite characteristic of the adult type, and the later changes which occur externally are, save in the development of the external organs of reproduction, of little significance in the future life history of the lobster. There are some points, however, which may be noted, not with reference to any definite stage-change, but with reference to the future development as a whole. Among these changes may be noted the increasing difference between the relative size of the eyes and body. In the first larval stage the eyes were very large and prominent, but in the course of later stages they become less and less prominent until in lobsters of great age and size, weighing ten or twenty pounds, the eyes are frequently no larger than shoe buttons. There is, moreover, a gradual thickening in the body of the lobster as the age increases. In the male lobsters this thickening occurs in the region of the cephalothorax, while in the female it is noted in the broadening of the abdomen which appears to widen for the accommodation and protection of the eggs which are borne under the "tail." There is also evident a gradual thickening and strengthening of all the body appendages, which, in the fourth stage are relatively rather light and frail.

Fifth Stage.

The fifth stage lobster is still too young to manifest these changes in any appreciable degree, either in form changes or in sexual differences. It is very often possible, however, to distinguish the fifth from the fourth stage lobster, although the differentiation depends upon very minor characteristics. In the fourth stage the basal segments of the first pair of antennæ are, when viewed in the dorsal aspect, well concealed beneath the margin of the projecting rostrum. In the fifth stage there is usually a greater prominence in the position of these basal antennal segments, so that they may be seen in dorsal aspect projecting from beneath the anterior portion of the rostrum border. (Plate XI.) The peak of the rostrum itself, moreover, undergoes a slight series of changes which occur fairly constantly in the development through the fourth, fifth, and sixth stages. These changes may be best noted by a glance at the diagrams in plate XX. The average length of the fifth stage lobster is 15–16 mm.

Sixth Stage.

By any differences in general morphology it is difficult to tell the sixth stage lobster from the fifth, since the general body form and appearance of the appendages is very similar in both stages. The chief point of difference lies in the fact that, whereas in the fifth stage lobster the left and right claws or chelæ were exactly similar in appearance, in the sixth stage there is in a large number of cases the beginning of a differentiation into the characteristic "nipping" and "crushing" claw, the former normally upon the right, the latter on the left. (Plate XIX.) This change comes about through a widening of the "crushing" claw, while the right, or "nipping," claw retains a close resemblance to that of the preceding stage. In some cases this claw differentiation does not occur until the seventh stage.

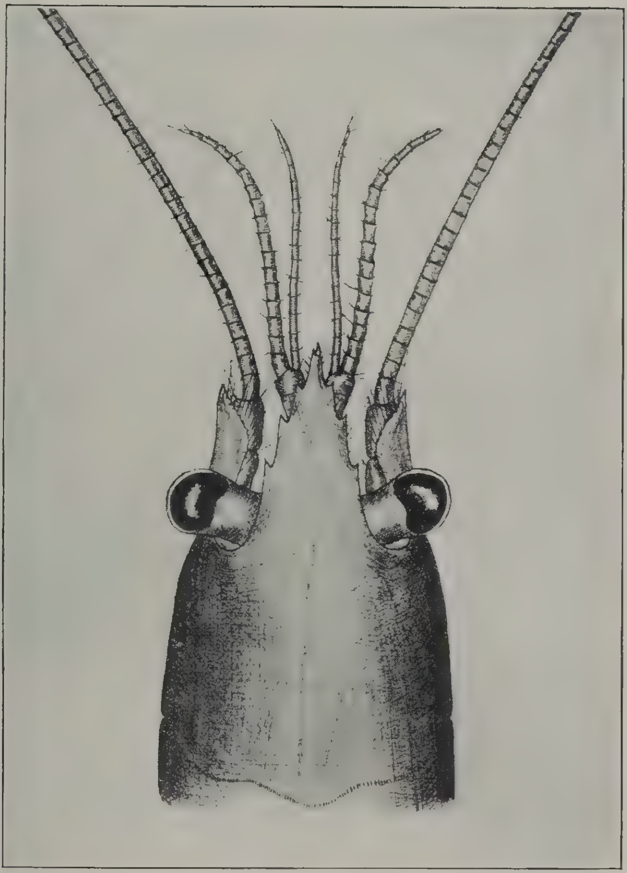

PLATE XI.—Head of young lobster in the fifth stage; shows the basal joints of the first antennæ projecting from beneath the anterior border of the rostrum, different in this respect from the condition in the fourth larval stage. (Drawn from life.)

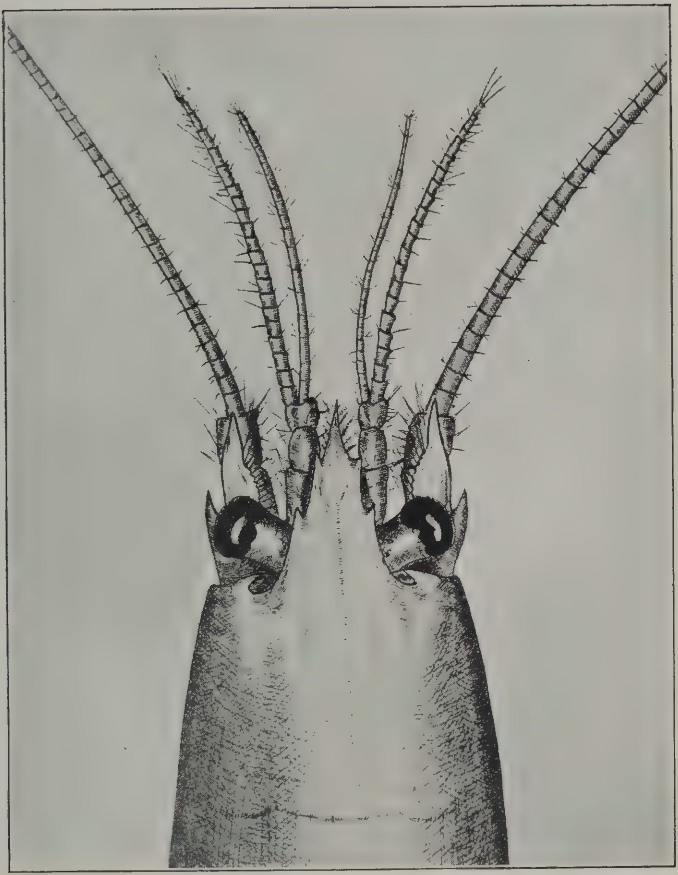

PLATE XII.—Head of young lobster in the sixth stage; presenting an appearance very similar to that of Plate V. Drawn from life.)

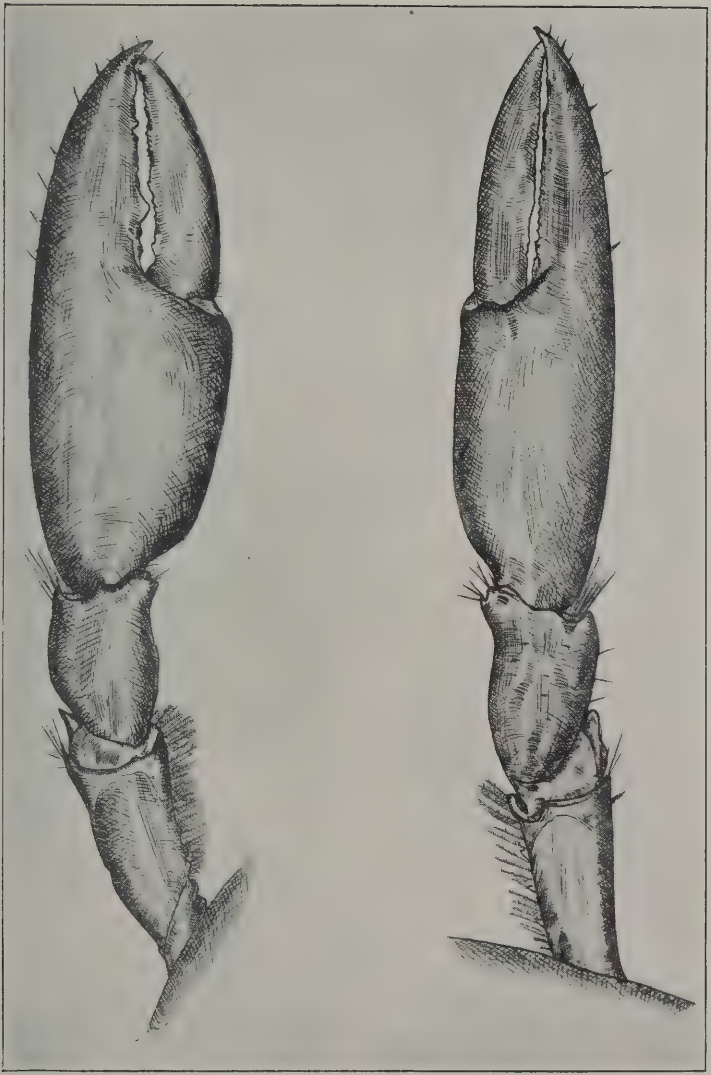

PLATE XIX.—Showing the difference which can usually be observed in the large claws when the lobster has entered the sixth stage. The left will develop into the "crushing" claw; the right, into the "nipping" claw. (Drawn from life.)

but such instances are not numerous, so that the change may be called most characteristic of the sixth stage lobster. (Plate XII.)

The same facts hold true in regards the appearance and development of the external reproductive organs. Very often these may be observed in the sixth stage as small buds upon the under side of the first abdominal segment. They may not, however, appear until the seventh stage, and even then they are seldom developed beyond the bud stage. The differentiation of the sex of the young lobsters can not be made clear by this means until the eighth or sometimes the ninth stage. The sex, however, can be determined earlier by observations of the openings of the egg or sperm duct upon the basal joint of the first and third ambulatory appendages, respectively.

Seventh to the Twelfth Stages.

The changes in form, if any, which may occur in the course of development from the seventh to the twelfth stage are too slight to be determined by any method yet devised. It is entirely possible, however, that continued observations may reveal some point which may be taken as a constant stage-criterion for these later stages. And yet, on the other hand, it is quite probable that, so far as the greater number of discriminating facts is concerned, the rapidity of assuming structural conditions which might be characteristic of a certain stage is much dependent upon the strength or vitality of the individual lobster and upon the conditions of its environment. The young lobster, with few exceptions, assumes the adult color type when it enters the eleventh stage of its existence. The question now arises whether or not there is a definite stage in which the young lobster assumes its adult structural form; or is there a definite stage wherein it arrives at an adult functional condition. It is quite probable that further observations may demonstrate the truth of the former hypothesis,* while in regards to the latter, the development is without doubt more strongly affected by environmental circumstances, it being probable that the sexual maturity is not reached before the fourth or fifth year of the lobster's life. Thus, with the scant data in hand, no facts can be positively asserted with reference to the change in form between the seventh and twelfth stages. The average length of the lobsters for these later stages is as follows:

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* Observations at the present date would indicate that the adult structural type is reached in the ninth stage.

5th stage	15½ mm.
6th stage	18½ "
7th stage	22½ "
8th stage	26½ "
9th stage	32 "
10th stage	38 "
11th stage	43 "

II. THE PIGMENTATION OF THE LOBSTER AND THE COLOR CHANGES IN THE SUCCESSIVE STAGES.

The study of the coloration in members of the class Crustacea, owing to the beauty and great variation in color, and to the physiological importance of the question of its nature, development, and function, has at all times proved an attractive field for the biologist. Yet unfortunately, to within a few years at least, it has been a field too fully overrun with speculation and too wholly unfrequented by the direct experimental results which can be gained from investigation upon many forms of both lower and higher crustacea. No doubt the desire to discover in many observed results the phenomena of natural selective tendency, in order to explain thereby the value and constancy of certain color markings and variations, has been responsible, in some measure at least, for the infrequency of definite lines of experimentation upon the conditions of color variation. When such experiments have been made, however, as in Cunningham's investigations upon the color of young flounders, environmental conditions have been found to possess a vast influence.*

It is not, however, within the intended scope of this paper to put forth the results of extended experimentation, but to give briefly a survey of some of the more common facts of color variation and pigment development in decapod crustacea, together with a preliminary report of a series of observations extending over eleven stages of *Homarus Americanus*—a series which includes the successive changes in color and coloration from the time of hatching to the attainment of the adult color type.

In the early stages of the lobster are found frequent and very wide range of color variation. This may occur as successive color changes from stage to stage, as variation in the color of different individuals in the same stage, or again, in the changes in color through which a single lobster may pass during a single stage. In the first larval stages these variations occur as rapid and transitory, and yet uniform, changes from one color to another. In the young adult forms, however, although a wide difference in individual color is manifest, the color and color pattern appears more permanent and more constant to a given type when this type has been once established, while the sudden, transitory changes so characteristic of the larval stages are entirely absent.

NATURE OF THE PIGMENTS AND OF THE CHROMATOPHORE SYSTEMS.

The pigmentation of the lobster may be resolved into three different constituents, the blue, the red, and the yellow. The blue is a soluble pigment, probably a lipochrome, dissolved in the blood, while the red and the yellow pigments, which may be also lipochromes, exist as a granular substance in certain cells, the chromatophores. Each one of these chromatophores is a granular cytoplasmic body of neuroglia or stellate shape, having a center from which branch thick trunks, dividing later into finer ramifications of a more or less tubular appearance.* In such cells the red or the yellow pigment lies, sometimes expanding far out into the small branches, again contracting into the center, where it remains evident only as a small dot of color. In cases of the greatest distention of these pigment cells, if they be thick in a green area, a homogeneous red coloration is produced. If in fewer numbers, each individual chromatophore with all its branches is plainly visible. In the early larval stages of the lobster it seems that the blue, soluble pigment is to be found at nearly all times, and when the red coloration is predominant it is merely because the blue color has been veiled, so to speak, by the great expansion of the red chromatophores, which both numerically and in comparative size are superior to the yellow. As stated, the blue pigmentation is diffuse. The chromatophores, however, are scattered irregularly, but often in regular groupings over the body and appendages, lying for the most part in the skin or cuticle or just below it. The distinction must be made, however, between the pigmentation of the chitinous exoskeleton and the pigmentation of the sub-adjacent epidermis in which the chromatophores reside and from which the pigment appears to be given off to the outer shell.† In the adult lobster the chromatophore-containing epidermis is quite concealed by the thick, calcerous exoskeleton which has usually, at this stage, absorbed a large amount of lime-salts. In the earlier stages, however, whose exoskeleton is composed of thin, transparent, chitinous substance, the colorings of the epidermis readily show through and continue to do so until, as the stages advance and more lime-salts are added to the shell, it soon becomes translucent and later opaque.

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* Young flounders having been kept for some time in tanks in the bottom of which mirrors were placed, showed in many cases large pigmentation of the under side, which is usually white; which seemed to show that some external cause, as light, was responsible for the change.

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* Keeble and Gamble: The Color Physiology of Higher Crustacea.

† It is readily observable by removing a bit of the shell of an adult lobster that sub-adjacent to each prominent olive-green spot lies a dense group of red pigment cells.

II. Groupings of the Chromatophores.

The distribution of pigment, including both the diffusion of the blue and the arrangement and grouping of the color cells, appears to be fairly uniform in the first three larval stages of the lobster. A slight variation may often appear in the third larva, however, namely,—the development of green blotches along the abdomen, thus somewhat anticipating certain characteristics of the fourth stage.* Generally speaking, the yellow pigment cells are not numerous, and have no regular arrangement either as individuals or as groups. Of the red chromatophores however, six fairly constant and well-defined groupings may be considered. (Plates XXIII, XVII.)

1. On the dorsal surface of the carapace in areas posterior and lateral to the region of the stomach.

2. Along the ventral and lateral border of the carapace where one complete row surmounted by a few irregularly placed cells is to be discerned.

3. In well-defined groups or frequently (especially in the third stage) in large individual cells, occurring in the dorsal region of the first three abdominal segments, close to and on either side of the mid-line.

4. In a variable area about the mid-anterior region of the last abdominal segment, where the chromatophores are often smaller and more scattered.

5. In the thoracic appendages on the segments nearest the body.

6. Throughout all segments of the chelipeds, especially in the third stage.

The groupings, or "chromatophore centers," as termed by Keeble and Gamble,† somatic, neural, visceral, and caudal, seem to have but little significance for color-distribution in *Homarus*, where in the internal organs are not highly pigmented. Several varieties of color may be produced, however, by the general color of the liver, alimentary tract, glands, etc., which in the comparatively transparent early stage lobsters may show through the delicate skin and produce colorations, the character of which depends largely upon the food of the young larvæ.

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* This olive green was not noticed in any of the preceding stages and was quite different from the lighter yellowish green which was, in those stages, very prominent on the sides of the carapace and abdomen.

† Keeble and Gamble· Color Physiology of Higher Crustacea.

III. Function and Behavior of the Pigments in Larval Stages.

The question of the possible function of these pigments in the lobster, as well as in other forms of crustacea, has yet to find a solution; likewise the question to what the contraction and expansion of chromatophores is due. Pouchet, in his work, "Changements de Coloration sous l'influence des Nerfs," gives the result of his experimentation upon such forms as the shrimp (Palæmon), with backgrounds of black and white. He finds that a black background, in sunlight, causes expansion, and that a white background, under the same conditions, causes contraction, of the chromatophores. He concludes that the background regulates the action of the chromatophores through the medium of the nervous system, and believes that this phenomenon is a case of protective or adaptive coloration. Other investigators maintain that the chromatophore pigments are merely a functionless product of metabolism. If we consider that a similarity in the color of the individual and that of the environment is a phase of protective coloration, surely experiments upon *Homarus* hardly uphold the theory of a protective function in the color cells; for in strong light the pigments are the brightest, and in the dark the young lobsters are most pale. It does seem, however, that there may be both protective and adaptive significance in the later stages of the lobster which do not show the discontinuous variation which is characteristic of the larval stages.*

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* Even in the adult lobster, however, whose dark, mottled olive brown and olive green adapt him for a life at the bottom of the sea among the rocks and red and green algæ, the phenomenon is probably the fortunate result of chemical influences; for, when placed in shallow water and exposed to the sunlight for some time, he readily becomes light colored again owing to the change which takes place in the pigments of the calcerous exoskeleton. In this case the light-

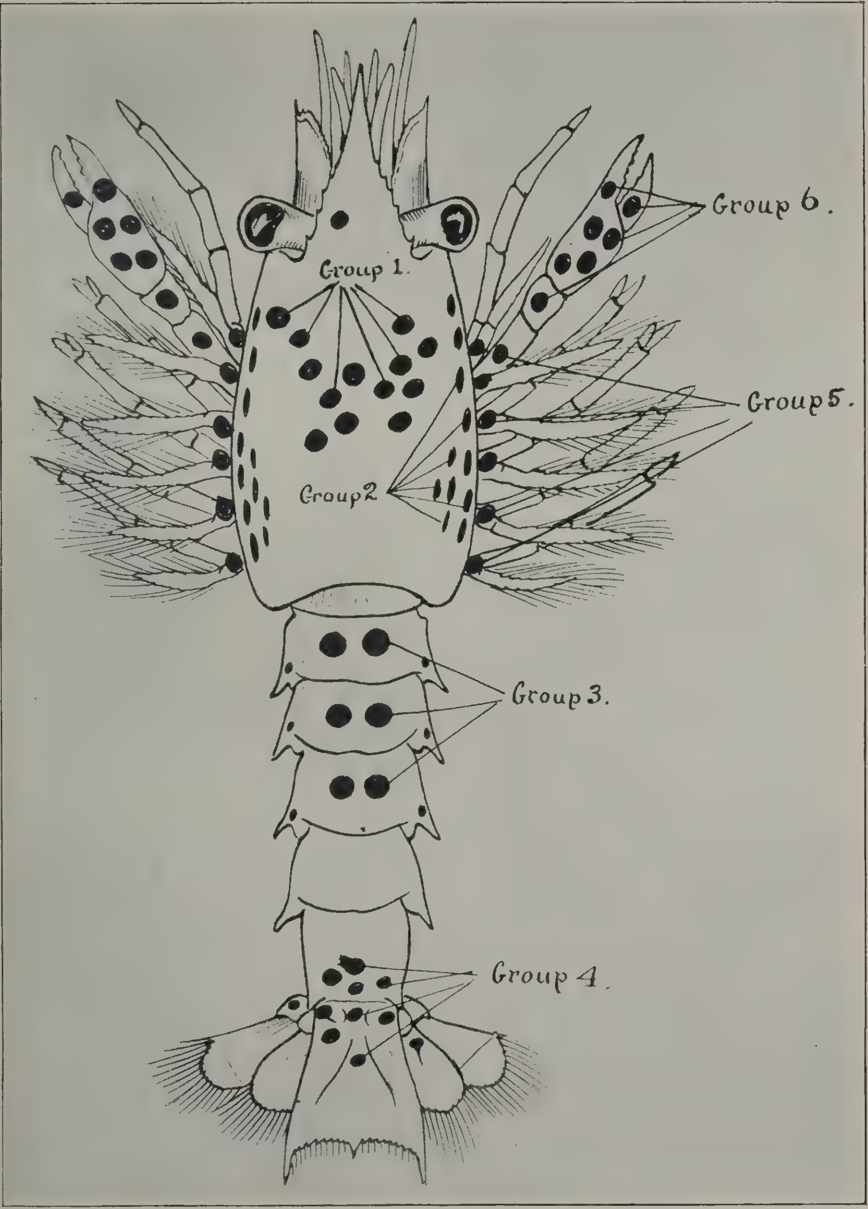

PLATE XVII.—Diagramatic representation (dorsal view) showing the arrangement of chromatophore groups common in the *first three larval stages*. The type drawing is of a third stage lobster, and shows also the arrangement of the swimming appendages. (Outline drawing from life.)

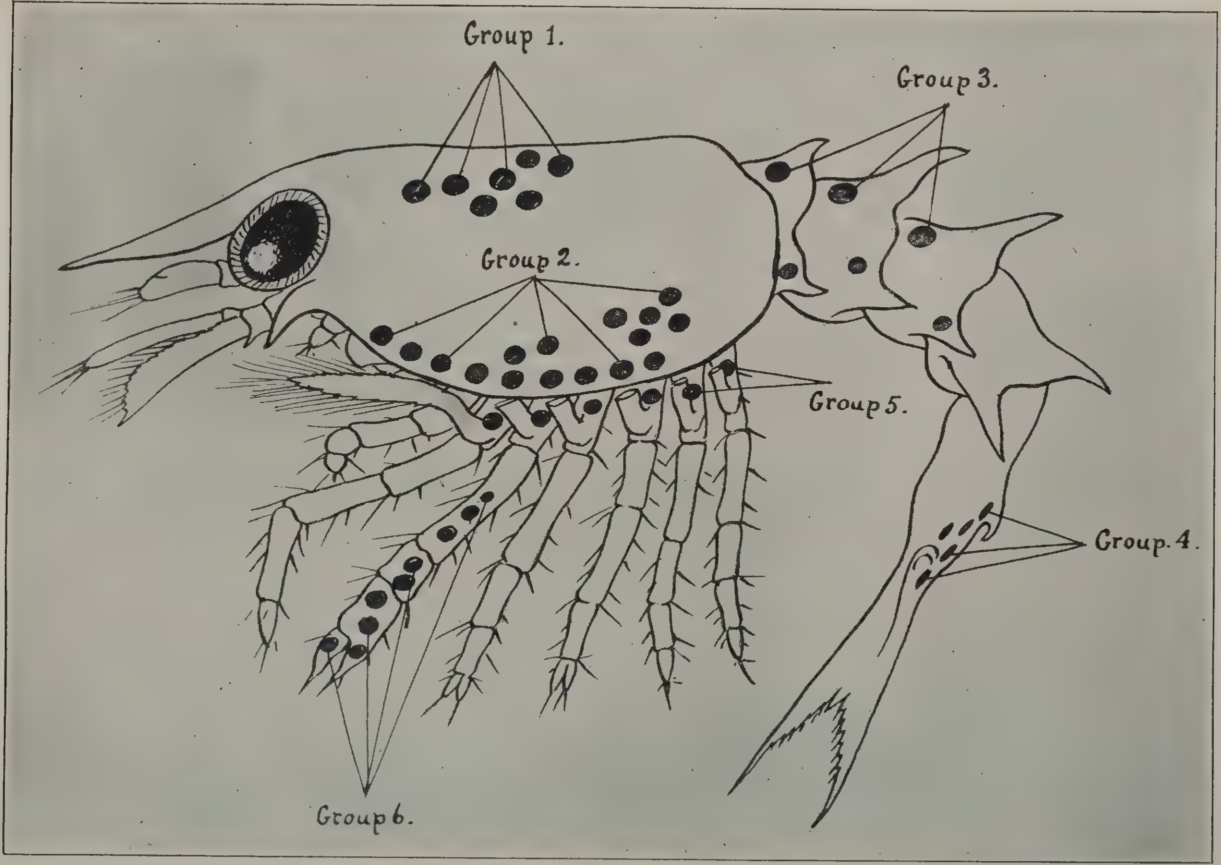

PLATE XXIII.—Diagramatic representation (lateral view) of the various groups of red chromatophores as commonly distributed in the *first three larval stages.* The type drawing is of a first stage lobster; the swimming appendages are represented as if removed. (Outline drawn from life.)

As a basis for observations upon the nature of the discontinuous variation of the early stages, experiments were tried which seemed to demonstrate that, while the yellow pigment cells are slightly influenced by light and heat, these stimuli tend to produce a marked expansion of the red chromatophore systems. Darkness artifically brought to bear upon large numbers of first and second stage larvæ in which red pigmentation was developed especially well resulted, with very few exception, in the contraction of the pigment into the chromatophore centers and in the regaining of the homogeneous blue coloration due to the soluble, diffuse pigment in the blood. Although in many cases the expansion of the red chromatophores followed as a result of putting the pale blue lobsters in the bright light, these results were less constant in occurrence and the changes required a longer period. Nightfall and sunrise appear to be signals for a change in the color of the first three larval stages. Those observed in the daytime, if the light was bright, were, as a whole, well pigmented with red, while those observed at night showed the chromatophores in a greater state of contraction. This diurnal flood and nocturnal ebb of pigment is characteristic of many of the higher crustacea.†

First and second stage larvæ, which were starved for some time, seemed to present red pigmentation to a greater degree. Repeated electrical stimuli also, in the majority of instances, gave similar results. Both of these facts would be in accordance with the theory that the occurrence of red pigment and the extension of the red chromatophores is due, or, to say the least, is associated with periods of weaker physical condition. This theory is partially substantiated by the fact that lobsters of all stages, from old adults down to the

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sensitive chromatophores of the pigment-producing epithelium do not appear to be the agent of adaptation to surroundings; indeed, it is very doubtful if the chromatophores themselves undergo any marked change due to penetration of light through the thick, calcerous exoskeleton of the adult lobster. Thus if we assume that the phenomenon of protective or adaptive coloration is manifested in the adult lobster, the principle involved in the color change is very different from that attributed by Pouchet to the protective color changes—"chromatic function"—which he found manifested in many young larval crustacea—changes which he believed to be brought about through the medium of the nervous system and its action on the chromatophore centers.

† Keeble and Gamble.

first stage, are much more active at night, as evidenced by the splashing and beating within the cages of the adult lobsters, which by day lie quietly at the bottom; or by the restless crawling of the young adults among the pebbles and shells of their glass dishes, as soon as night has fallen; or by the more active swimming of the young larvæ at night about the glass cylinders in which they were observed.

To the results of many of these experiments appeared some contradictory facts, as for example,—on a dark and cloudy day vast numbers of young larvæ swimming about the bags evinced red pigmentation to a high degree. Not only this, but in the case of many large adult lobsters, floating in cages at the surface where they were exposed to the direct sunlight, their red coloration to a large degree was lost and the color became a brilliant blue often variated by leopard spots or mottlings. The normal color was not regained after some specimens had been sunk to the bottom of the harbor for a period of three weeks. It should be noted, however, that this change was not due to a disturbance of the chromatophores, but to some chemical change in the pigments of the exoskeleton.

IV. Pigmentation of the Fourth and Later Stages.

Fourth Stage.

As has been stated on a previous page, when the lobster moults into the fourth stage there may be a wide variation in color and color patterns, but when once assumed there is, with a few exceptions, a constancy to this type throughout the stage, and often enduring into successive stages. The phenomenon of rapid and transitory color changes so characteristic of the first three larval stages is no longer present.

In the color scheme of this stage we may note three varieties: (1) yellow, (2) red, (3) green. These terms designate the color types in which the stated color is *predominant*, but in which there may be many modifications; for instance, a yellow lobster may and usually does show in certain areas no small amount of red pigmentation, and in other areas, green or orange. Likewise the type designated "green" is rarely wholly green, but shown many areas of red, brown, or yellow. The red type is the *only one* which may be found alone and constant without other color modifications with yellow, green, or brown. Such manner of constant red pigmentation may extend, with no change, through several or many successive stages of development. These other variations in color may occur, designated in terms of yellow-green, green and red, or reddish-brown, orange-red, but are to be considered as *modifications* of the *three main types.*

The pigmentation of the fourth larval stage manifests some points which link it to the third, especially the pronounced red of the chelæ, the grouping of red chromatophores as in group (4), and the predominance of the olive-green along the sides of the abdominal segments. The coloration during the latter part of the fourth stage, moreover, anticipates to some extent the color of the succeeding stage, so that it is not at all uncommon to find lob ters in the late fourth stage which show a deepening brown color, wherein there may be seen dimly light spots on various parts of the body and appendages, anticipating again, in this feature, a peculiarity of the next, or fifth, stage. Such anticipation of color is fairly common in the latter part of this and later stages as the time of moulting draws near.

COLOR CHANGES IN THE LATER STAGES.

As has been already stated, in the fourth and later stages there is never found the rapid and transitory color changes characteristically exhibited in the first, second, and third larval stages. There usually is, however, some slight color change between the beginning of a certain stage and the end of the stage—a change which is generally manifested as a darkening of whatever the color type may be, and in a loss of detail in the color markings or patterns. This fact is one which, developed at this period, holds true for all later stages, namely *a darkening in color as the time of moulting draws near.* In spite of this general fact there may occur a more or less sudden change in the color of these later stages, although such cases are very rare indeed. I have recorded a case wherein a lobster of the eighth stage whose color was *cream-slate,* being observed two days later, manifested a marked *salmon* color. The lobster had not moulted between the time of the observations, and moreover the salmon color persisted through the eighth and ninth stages, at the end of which time the observations were unfortunately brought to a close by the oncoming winter season. Thus, in the following stages, we may expect to find a decided constancy manifested in adherence to a definite color pattern, easily observable in the same lobster through many successive stages. As has been noted, this fact is shown especially well in the development of "*red lobsters,*" though it is well demonstrated by observations made on many other varieties more common.

It may be here stated that methods devised at the Rhode Island Fish Commission Hatchery at Wickford for rearing the young adult lobsters past the fourth stage furnished excellent facilities for making observations upon the changes in form and color in the later stages, each individual lobster whose definite age and stage was known being confined in its own separate apartment where observation might be carried on from day to day. Insomuch as awnings were raised over the cars containing the young lobsters, which occupied a rather superficial position in the water, it is quite probable that normal conditions of environment were produced and that none of the color variations observed were due to the effect of bright sunlight, which, as was demonstrated in some cases, was responsible for certain color changes in a large measure. The observations upon all the stages were made while all the lobsters were under the same conditions.

Fifth Stage.

The characteristic color of the fifth stage lobster is a rich brown set off by light spots varying (except in the case of the body spots, which are always white) in intensity from snow white to dirty yellow, often marking definite muscle attachments, yet not infrequently

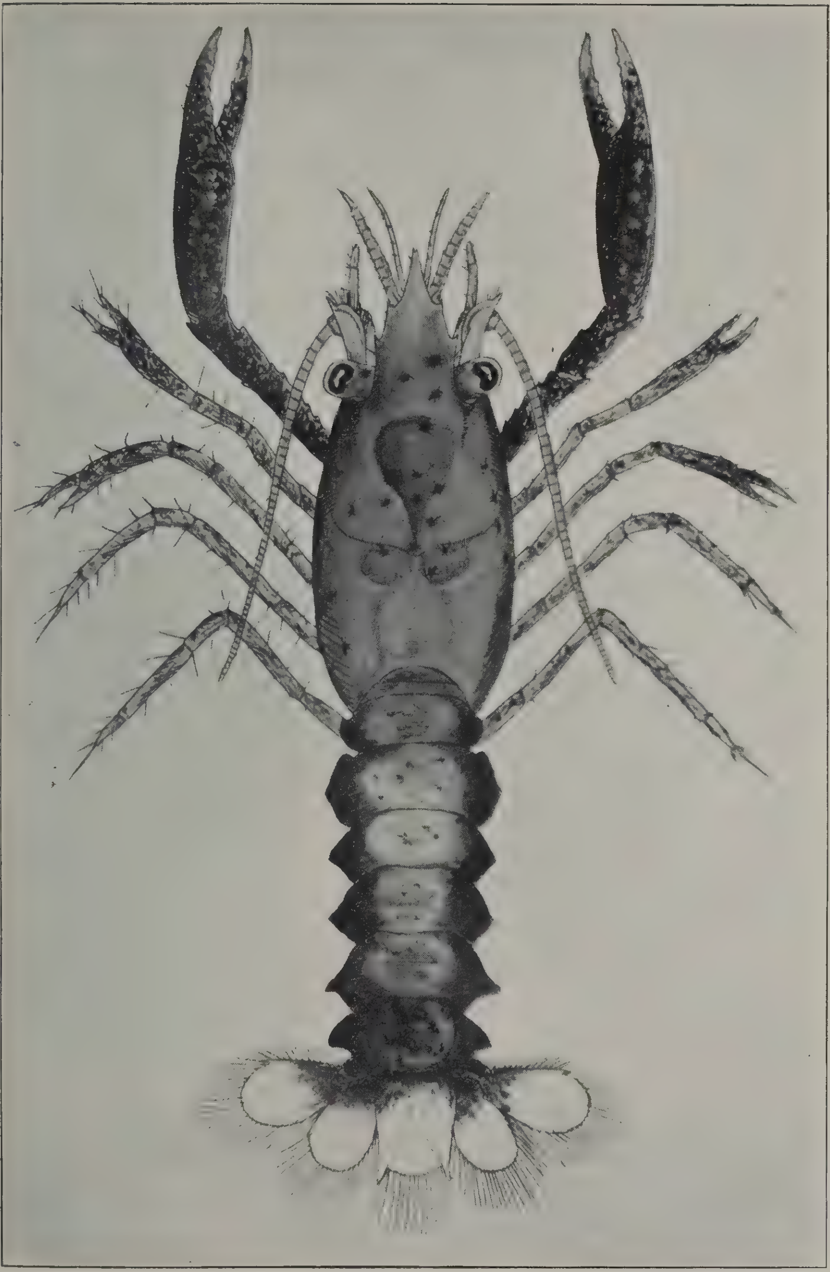

PLATE XVIII.—The fourth stage lobster, showing the loss of swimming appendages which has ken place since the third larval stage. (Photograph from a drawing in color.)

occurring in varying localities of the body and appendages. This typical brown may, and usually does, have many modifications among which the most prominent are red-brown, maroon, and brown and green. The light spottings may occur in the following localities: tips of chelæ, of maxillipeds, of telson, of rostrum, of pleura, and tips of the exopodites of the last pair of abdominal appendages; or they may exist as body spots. In case of the last, the position is usually on the anterior and lateral region of the carapace, where they mark certain attachments of the gastric or mandibular muscles, or they may form large light patches over the heart or gastric region of the carapace. The spots may occur in any or all of these positions at once, but the most constant are the claw tips, body spots, and those on the first abdominal segment and on the telson.

It may here be noted that the transparency of the body often so evident in the larval stages, wherein many of the internal organs such as the green gland and vesicle, stomach, intestine, heart, liver, gills, etc., could be demonstrated more or less distinctly, and which has to a great extent been lost in the fourth larval stage, has in the fifth stage disappeared to a still greater degree, so that it is with difficulty that the position of the above mentioned organs in the body cavity can be discerned; hence, in this and the later stages the color variations are due to the cuticular and epithelial pigmentation alone, or to changes in the pigment of the exoskeleton. I am informed by Mr. E. W. Barnes, whose observations upon the color variations in the ninth and eleventh stages have materially helped in producing data, that he came upon a lobster which, soon after moulting into the tenth stage, manifested a remarkable transparency in all parts, so that the action of the heart and position of the gills could be plainly observed through the carapace, which was of a bluish color.

Sixth Stage.

The color of the sixth stage lobster resembles very closely that of the fifth stage. Indeed, by examining the colorations it is almost impossible to tell the two stages from one another by this method.

One fact, however, is noticeable: the light spottings in the sixth stage are usually more numerous and varied than in the fifth. In the sixth the telson markings often assume the form of bands or borders of white, evident also on both segments of the lateral appendages of the last segment. These bands were sometimes rust colored, but this variation was more often evident in the seventh stage.* In the fifth stage the white spottings were seldom seen on the pleura of more than the first abdominal segments; in the sixth stage they often were observable on the pleura of the first, second, and third somites. Bands or streaks of white were also often evident upon the posterior border of some segments of the chelæ. It was noted that during the latter part of the sixth stage there was some tendency to anticipate the seventh stage, the color frequently changing to a dark drab.

Seventh Stage.

The color of the seventh stage lobster is usually so radically different from that of any of the preceding stages that it can be recognized with little difficulty. With hardly an exception in the many individuals examined, the characteristic color was *pure slate*, becoming gradually darker as the stage advances, having the modifications, *blue-slate*, *green-slate*, and *cream-slate*. The white spottings are full as frequent and quite as constant as in the sixth stage; the only difference being the tendency for the spots to become a cream color, or yellowish, rather than snow-white, which seemed more characteristic of the fifth and sixth stages. The following notes, taken from the records of ten successive cars containing individual seventh stage lobsters, show the constancy of this color characteristic:

Spec. 1. Moulted Aug. 1. Color, slate; claw tips, light, but not white; body spots, light; border of telson, yellowish white.

Spec. 2. Color, slate, somewhat bluish; body spots and others not prominent.

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*It is entirely possible that this *rust color* may have been due to foreign matter from the wire of the cages.

Spec. 3. Color, greenish slate; claw tips, cream; body spots, white; telson of homogeneous color, slate. Aug. 26. Color changing to a more pronounced drab.

Spec. 4. Color, dark slate, claw tips and body spots, white; white borders on outer segments of last abdominal appendages.

Spec. 5. Color, slate; somewhat mottled; telson border, ends of claws and ends of maxillipeds, white or cream color.

Spec. 6. Color, slate; claw tips, snow-white; body spots, light; telson border, cream color; anterior cephalic body spots light.

Spec. 7. Color, creamy slate; claw tips, cream color; border of telson, rusty; body spots, light.

Spec. 8. Color, slate; claw tips, white; telson border, cream color; white patch over region of heart.

Spec. 9. Blue slate color; claw tips, rusty cream; body spots, light; telson one homogeneous color.

Spec. 10. Color, slate; claw tips, cream color and body spots white; border of exopodite (last abdominal segment) and border of telson, cream color.

The following observations upon forty-nine seventh stage lobsters give some notion of the general color characteristics of the stage. All the observations recorded in this table were made very soon after entrance to the stage. For convenience in comparing this general color plan of the seventh stage with that of the later stages, tables referring to the eighth, ninth, tenth, and eleventh stages are also here appended. Reference to them will be made on a later page: (The numbers represent percentages.)

COLOR.	7th.	8th.	9th.	10th.	*11th.	*12th.
Pure slate	77	18	10	......	......	......
Blue slate	4	35	6	13	......	......
Cream slate	......	25	13	......	......	......
Green slate or green blue	12	4	13	7	......	......
Blue	......	....	37	15	20	......
Olive green	......	1 spec.	....	30	40	......
Olive red-brown	......	....	....	15	40	......
Mottled lobsters	......	....	1 spec. in 30	54	100	......

* The number examined was too small to give valuable results by percentages.

The following tables show the color of many lobsters in the seventh, eighth, ninth tenth, and eleventh stages which were observed in almost every case within four to twelve hours after moulting. The terminology used to designate the colors is somewhat crude, but an effort will be made at a later date to exchange for these terms those of a standard color chart:

7*th Stage.*	8*th Stage.*	9*th Stage.*	10*th Stage.*
Slate.	Deep blue slate.	Gray.	Olive green, mot.
Slate.	Blue slate.	Rusty slate.	Light blue slate.
Greenish slate.	Dark slate.	Blue.	Olive and blue.
Slate.	Creamy slate.	Blue.	Olive green.
Slate.	Blue slate.	Blue slate.	Olive and red brown.
Slate.	Dirty slate.	Blue.	Olive green, mot.
Slate.	Slate.	Blue.	Green slate.
Slate.	Cream slate.	Brown slate.	Dark slate, mot.
Green slate.	Blue slate.	Green slate.	Blue slate, mot.
Blue slate.	Cream slate.	Cream slate.	Blue, mot.
Slate.	Cream slate.	Blue.	Light blue slate.
Slate.	Light brown.	Light blue.	Blue, mot.
Slate.	Brown slate.	Cream slate.	Green and Brown, faint mot.
Red slate.	Cream slate.	Blue.	

7th Stage.	*8th Stage.*	*9th Stage.*	*10th Stage.*
Green slate.	Olive.	Light blue.	
Blue slate.	Slate.	Cream slate.	
Slate.	Cream slate.	Slate.	
Slate.	Blue slate.	Very dark slate.	
Slate.	Slate.	Deep blue slate.	
Slate.	Cream slate.	Slate.	
Green slate.	Blue slate.	Dark slate.	
Slate.	Cream slate.	Blue.	
Green slate.	Cream slate.	Blue.	
Slate.	Blue slate.	Green slate.	
Slate.	Cream slate.	Light blue.	
Slate.	Blue slate.	Salmon.	
Slate.	Cream slate.	Metallic blue.	
Slate.	Brown slate.	Slate.	
Slate.	Light brown.	Cream slate.	
Slate.	Slate.		
Slate.	Green slate.		
Slate.	Brick red.		
Slate.	Blue slate.		
Slate.	Green blue.		
Slate.	Slate.		
Slate.	Blue slate.		
Slate.	Blue slate.		
Slate.	Blue slate.		
Slate.	Blue slate.		
Slate.	Light blue slate.		
Slate.	Slate.		
Slate.	Dark slate.		
Slate.	Blue slate.		
Slate.			
Slate.			
Slate.			
Slate.			
Brown slate.			
Brown and green.			
Green slate.			

11th Stage.
Olive green, mot.
Olive green, mot.
Blue, mot.
Olive and brown, mot.
Olive and brown, mot.

Eighth Stage.

In color markings the eighth stage is very similar to the seventh; but one point can be noted where there lies a difference and this only when many specimens are examined, for a single individual of the eighth stage can not, by its color, be told from the seventh. In the seventh stage the number of modifications of the characteristic color, slate, were comparatively few, the greater number of specimens examined remaining constant without merging into the blue-slate cream-slate, or red-slate. In the eighth stage the comparison of color statistics readily shows that the number of variations, or modifications of the slate color is very great and that there is a marked tendency to develop green-slate, brown-slate, and especially *blue-slate* and *cream-slate.* A description of ten individuals of the eighth stage, taken at random, shows the general characteristics of markings for individuals, while the foregoing table for the eighth stage gives an approximate notion of the general scheme of colorations for the stage as a whole.

Spec. No. 1. Color, deep bluish slate; claw tips, rust color; also border of telson. Body spots not readily discernable.

Spec. No. 2. Very dark slate color; claw tips, cream; body spots, white; telson border, rusty cream color.

Spec. No. 3. Color, cream-slate bordering upon bluish; claw tips, cream color; with the very tips rusty; body spots very distinct; border of exopodite (last abdominal segment), very light.

Spec. No. 4. Color, bluish slate; claw tips, rusty cream color; margin of telson, rust color; no body spots evident.

Spec. No. 5. Bluish slate showing a metallic luster; body spots, light, but not white; lighter over stomach region.

Spec. No. 6. Light bluish slate color; tips of claws, cream; body spots, faint; telson, homogeneous coloration.

Spec. No. 7. Color, cream-slate; claw tips, cream color; border of telson, rust color; body spots, light.

Spec. No. 8. Color, cream; body spots, white; claw tips, cream; number of white spots on and posterior to cephalo-thoracic line.

Spec. No. 9. Color, cream-slate; claw tips, white; border of telson and of exopodites, cream color; body spots not prominent; color changed very suddenly to salmon.

Spec. No. 10. Eccentric coloration in all details; general color, light brown; outer claw tips of each chela, cream color, with band of same along whole margin of claw; left chela has outer claw very white, both on tip and outer margin. Both of the exopodites (of last abdominal somite), cream color; endopodites of same, light; body spots, snow white; whole lobster quite transparent.

Ninth Stage.

The difference in color between the ninth stage lobster and the stages which immediately precede and follow it can be determined only by viewing such general conditions as those which formed the basis of our observations on the color characteristics of the seventh and eighth stages, wherein the stage could never be determined by the color of the single individual, but which nevertheless held a characteristic that could be used readily enough as a rough criterion for the distinction of large numbers of lobsters whose exact stage was not known. So it is in the case of the ninth stage; when many lobsters of this stage are observed as to their color, the fact is evident that there is a tendency for the *blue* coloration which was beginning to be emphasized in the eighth stage to have still greater prominence here, with a corresponding diminution of the relative number of lobsters manifesting the pure slate so characteristic of the seventh stage, or the cream-slate and blue-slate more characteristic of the eighth stage. A glance at the table for the ninth stage will show these facts. In this stage the white spottings have begun to become less prominent and less frequent in occurrence.

Tenth Stage.

The fact of the gradual exclusion from stage to stage of certain color combinations from the general system of coloration of a definite stage-period manifested heretofore in all the previous stages also holds true for the conditions found in the tenth stage lobster. Here, it will be readily noted by a glance at the table, the number of slate and cream colored lobsters has greatly diminished. Blue, blue-slate, and green-slate, however, remain fairly constant in occurrence, while there has also been a tendency toward the development of an *olive-green* and an *olive-brown* combination. In the tenth stage, moreover, the light spottings are seldom observed we see, as the foregoing table denotes, traces of the *dark mottling* so characteristic of the adult lobster, a phenomenon which, with a very occasional exception, makes its *first appearance* in this stage.

Eleventh Stage.

Very unfortunately it was not possible to carry on observations upon many of the eleventh stage before the oncoming winter weather and the consequent necessity of sinking the lobster cars to the bottom of the harbor cut short all investigations. The meagre facts which can be reported upon the coloration of this stage may be briefly obtained from a glance at the table. *Pronounced mottling* seems to become a constant feature in this stage, and the few individuals examined show a greater resemblance to the color type of the adult lobster than does any previous stage. Indeed, it may be safely said that, in consideration of this fact, and that of the frequency of the olive and red-brown combination, the *adult system of color* is approximately reached in the eleventh stage of the lobster.

SUMMARY.

Changes of Form in Successive Stages.

The life of the lobster consists of a series of stages (the first four are called the larval stages) each of which represents a stage-period, the time between any two successive moults. The lobster grows by moulting, never between moults.

The changes which are undergone in the first four stages are the most distinct. After this period the changes in form are slight from one stage to another.

First Stage.

Owing to the much coiled position in which the young lobster lies in the egg, at the time of hatching this infolding of appendages and abdomen about the head is very apparent and endures for some little time; gradually, however, the parts extend and the appendages become functional. The characterisitic points of the first stage lobster may be briefly summarized as follows:

1. Dorsal surface of cephalo-thorax in lateral aspect forms a decided arc.
2. Eyes very large and prominent.
3. First pair of antennæ consist only of exopodites.
4. Second antennæ consist of exopodite and endopodite, the former very short and tubular, the latter broad and leaf-like.
5. The thoracic appendages have feathered swimming attachments (exopodites).
6. The body-origin of the thoracic limbs is posterior to position in adult type.
7. There are no appendages on the ventral portion of the abdomen.
8. The tail, after unfolding, consists of a simple "tail-fan."
9. The average length of the first stage is 8 mm.

Second Stage.

The external changes which mark the entrance to the second stage concern chiefly the changes which take place in the antennæ and in the abdominal segments:

1. Dorsal surface of cephalo-thorax not as convex as in first stage.

2. Eyes somewhat less large and prominent.

3. The inner branches (endopodites) are developing from the exopodites of the first pair of antennæ.

4. The endopodites of second antennæ increased slightly in length.

5. The thoracic appendages have shifted in respect to their body origin to a more anterior position.

6. The legs, chelipeds, and maxillepeds are still fitted with swimming exopodites.

7. From the under side of the 2nd, 3rd, 4th, and 5th abdominal segments have developed swimmerets.

8. Tail-fan of same appearance.

9. Average length of second stage is 9½ mm.

Third Stage.

The change manifested in the shifting from the second to the third stage also concerns chiefly the antennæ and the abdominal segments:

1. Dorsal surface of cephalo-thorax but slightly curved.

2. Eyes relatively smaller for size of body.

3. The inner branches of the first pair of antennæ about equal in length the outer branches.

4. The exopodites of second antennæ have increased slightly in length.

5. The thoracic appendages have undergone a further forward shifting.

6. The exopodites of the thoracic appendages are still functional.

7. The swimmerets of the abdomen have each developed a delicate fringe of setæ.

8. The last segment of the abdomen has given out on each side an appendage consisting of an exopodite and an endopodite, these fringed with setæ. The appearance of the "tail" has been greatly modified since the previous stage.

9. The average length of the third stage is 11 mm.

Fourth Stage.

The changes which occur between the third and fourth stage are the most distinct of any which take place in the life history of the lobster, and are as follows:

1. The body undergoes a straightening and elongation.

2. In the first antennæ the two branches of each appear equal in length, definitely segmented and bordered with setæ.

3. The second antennæ (the endopodites) emerge as long, segmented, whip-like structures which now, because of the prominence of the basal joints, can be folded back along the side of the body.

4. The thoracic appendages have again shifted forward, this time to *adult position.* Moreover, they have lost, with the exception of the maxillepeds, the swimming branches (exopodites). Rudiments of these are evident, however, as small functionless stumps with no sign of setæ on the ischium or third joint of the chelæ and ambulatory appendages.

5. The appendages on the under side of the abdomen have become larger and stronger and fringed with a heavy border of setæ. They become highly functional in this stage.

6. The exopodites and endopodites arising from the last abdominal segment and forming with the telson the "tail-fan" have developed in size and now are equal in length to the telson itself. They too are fringed with a heavy border of long setæ.

7. The average length of the fourth stage lobster is about 13 mm.; it is now almost the epitome of the adult lobster.

In the succeeding stages of the young lobster many of the changes which take place may be noted, not so much with reference to any definite stage as with regards to the continued development as a whole. Of these we may note the following:

1. The eyes become less and less prominent as the stages advance.
2. A broadening and thickening in the body occurs.
    - *a.* In the male this takes place in the cephalo-thorax.
    - *b.* In the female, the broadening is in the abdomen.
3. There is a thickening and strengthening of all the body appendages with a gradual variance in the appearance of the right and left chelæ.

Fifth Stage.

The fifth stage lobster is too immature to at once manifest many of these changes; the chief characteristic of this stage being the prominence of the basal joints of the first antennæ, as in contrast to this position under the rostrum border in the fourth stage. (Plate XI.) The average length of the fifth stage lobster is 15–16 mm.

Sixth Stage.

The point of distinction of the sixth stage is the beginning of a differentiation in the shape of the chelæ, the right manifesting a tendency to develop into the "nipping" claw, the left into the "crusher." This change sometimes is not evident until the seventh stage. The external reproductive organs often make their appearance in this stage. They first appear on the under side of the first abdominal segment. This phenomenon may, in some cases, be delayed until the seventh stage. The sex, however, can often be made out in the sixth stage by the position of the openings of the sperm ducts or oviducts, which appear on the basal segment of the first and third thoracic limbs, respectively.

Seventh to Twelfth Stages.

The changes in form which occur between these stages probably depend very much upon the vitality and general condition of environment of the young lobster. Thus the impossibility to attribute to any one of these later stages definite characteristics in form. The most that can be stated is that some features of development appear to be manifested at *about* a certain stage. Thus the external reproductive organs, if they do not appear in the sixth stage, do appear in the seventh. In this and the following stages the difference in the large claws becomes more and more evident. It may be said that the lobster assumes the adult structural type (with reference to external morphology) in the ninth stage of its existence.

PIGMENTATION AND COLOR CHANGES.

In the life history of the American lobster there occur very marked changes in color and coloration. These changes may be grouped under three heads, as follows:

1. The sudden transitory changes in color (from blue to red, and red to blue) which take place in the first three larval stages.

2. The more gradual changes in color which a young lobster, beyond the fourth stage, may experience between any two successive moults; *i. e.*, during one stage-period. These changes are characterized by a general darkening in color as the stage-period advances and the time of moulting draws near.

3. The very gradual, progressive change in color and coloration which takes place in the development of the lobster after the third stage, and especially between the third and the twelfth stages. This change is characterized by the slow assumption of mottled olive green and brown as the young lobster approximates to the adult color type.

The color of any lobster is due to three primary pigments, all of which are probably lipochromes:

1. A red pigment found in the stellate color cells or chromatophores which are very abundant of the lobster.
2. A yellow pigment located in the yellow pigment cells, or chromatophores, which are less numerous than the red color cells.
3. A diffuse blue pigment found throughout the body in the blood, in which this pigment is soluble.

These pigment cells are found in the epidermis or just below it in the pigment producing epithelium. A distinction must be made between the color of the epidermis, which, in the early stages shows through the thin chitinous exoskeleton, and the color of older lobsters which depends upon the amount and nature of the pigment absorbed by the thick, calcerous, and opaque exoskeleton.

Often the color of the lobsters in the early stages is modified greatly by the color of the internal organs, such as stomach, intestines, liver, glands, etc., which may show through the thin, transparent, and chitinous wall of the exoskeleton before it has taken up from the water a large amount of lime salts.

The chromatophores themselves are granular, cytoplasmic bodies of irregular stellate shape, having centers from which branch thick trunks, dividing later into finer ramifications; through these the pigment ebbs and flows. These cells are found in fairly regular groups over the body and appendages, the groupings remaining fairly constant through the first three larval stages (cf. p. 59).

The function of the pigments of the lobster or of the crustacea in general is not known. By various investigators they are held to be reserve products, or functionless products of metabolism, or again, to have a decided protective function ("chromatic function" of Pouchet). Protective function on the part of the chromatophores is, however, difficult to demonstrate, for the changes in the color of the young larvæ do not appear to be protective; and, moreover, the color and coloration of later stage lobsters, which certainly do manifest color adaptation to environment, appear to be influenced not so much by chromatophore activity as by chemical changes in the absorbed pigments of the calcerous exoskeleton brought about by the influence of light intensity or other environmental conditions.

Chromatophore activity may be stimulated by light, heat, and electricity, the resultant changes taking place most quickly in the larval stages. These stimuli cause, under most conditions, *expansion* of the chromatophore cells, while darkness and cold result in their *contraction.* These changes which determine the color of the young lobsters, as red or blue, result not only from the application of artificial stimuli, but, in the case of light, with the change from night to day. Light intensity, rather than backgrounds, seems to condition the ebb and flow of color pigment in *Homarus.*

The phenomenon of rapid, transitory color changes is lost when the lobster enters the fourth stage. This stage allows a wider range of color variation than any following stage. The characteristic color scheme includes *yellow*, *red*, and *green*, and the fourth stage lobsters exhibit some variety of modification or of mixture of these three main types.

The characteristic color of the fifth stage lobster is a *rich brown*, set off by light spots varying in intensity from snow white to dirty yellow. These spots may occur on the tips of certain appendages or on certain parts of the body where they usually mark the attachments of muscles. The body transparency has disappeared almost wholly in this stage; exceptions, however, may be noted.

The color and coloration of the sixth stage lobster is hardly distinguishable from the preceding stage. Bands and spottings of white are, however, usually more prominent in the sixth stage. During the latter part of the stage the color often changes to a dark drab, thus anticipating the stage succeeding.

The color of the seventh stage lobster is very typical and characteristic, *slate*, having as occasional variations, *green-slate*, *blue-slate*, or *cream-slate.* These modifications, however, are not common in this stage.

The individual eighth stage lobster can not by his color be told from the seventh stage. If large numbers are examined the characteristic stage color appears as *blue-slate* or *cream-slate.*

A difference between the eighth and ninth stages can be determined only by the observation of large numbers of individuals. Here it appears the characteristic color is *blue,* varying in its intensity. The white spots have in this stage become much less prominent and less frequent in occurrence.

The color of the typical tenth stage lobster is *olive-green.* The stage still contains many blue and also many olive and brown lobsters. The dark mottling in olive and brown makes its first appearance in this stage, while the white markings are no longer to be observed.

The eleventh stage of the lobster is characterized by the assumption in all individuals, of a *mottled olive* or *olive and brown color,* the general color type of the adult lobster.

PHOTOTROPISM IN THE LARVAL AND EARLY ADOLESCENT STAGES OF HOMARUS AMERICANUS.

By P. B. HADLEY.

Science, N. S. Vol. xxii, No. 569, p. 675, September 24, 1905.

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PHOTOTROPISM IN THE LARVAL AND EARLY ADOLESCENT STAGES OF HOMARUS AMERICANUS.

IN view of the interest which, by the recent excellent work of Keeble and Gamble on the color physiology of higher crustacea, has been renewed in the study of the effects of light upon many forms of littoral crustacea, the following results obtained during the past summer, in a series of experiments upon the effect of light on the larval and early adolescent stages of the American lobster, may be appreciated by some who are engaged in investigation of a similar nature upon other forms. The records of the following experiments cover but a small part of the field of inquiry into the effect of light upon these forms, in so much as they do not take up the subject of the influence of light upon chromatophore activity or pigment movement, but merely attempt to describe the reactions of the first five stages of *Homarus americanus* to light, upon backgrounds of black and white.

The apparatus used for the experiments consisted of an oblong wooden box whose inside dimensions were 6 x 18 x 4 inches (deep). The box was black inside and fitted with a light-tight cover, through one end of which protruded, to a length of 6 inches, a cardboard tube, 1½ inches in diameter, the function of this tube being to admit none but nearly parallel rays of light into one end of the box, thus distinctly localizing the light area. In cases wherein a white background was required, the black interior of the box was merely covered with white paper, as was the inside of the light-tight cover.[1]

The method of conducting a single experiment was essentially as follows: The box was filled with salt water to a depth of 2 inches or thereabouts, and placed in a quiet and level position. The desired number of lobsters, together with sufficient salt water to make the total depth about 3 inches were added. When the water had quieted and the young lobsters had arranged themselves more or less uniformly in the water area, the cover was placed in position. At intervals, varying from 5 to 15 minutes, the cover was removed and the position of the young lobsters was observed. After some of the observations the cover would be reversed in position, so that the illuminated area in the water would be changed to the opposite end of the box. After other observations, however, the cover would be left as in the first instance, or removed entirely until another uniform distribution in the position of the young lobsters had been obtained. Whether the position of the cover was changed or not, the results, with few exceptions, agreed with great uniformity.

The light intensity was regulated by the time of day at which the observations were made—either at noon, mid-afternoon or nearly evening. In this way, without using artificial means, it was possible to regulate the degree of light intensity with a very fair degree of precision. In the case of studying monochromatic lights (red and blue) sheets of glass were used, the plate being placed over the entrance of the tube. Without doubt, liquid filters would have been an advantage; but the experiments which were made with glass plates gave such marked and definite reactions that they were judged satisfactory for preliminary work.

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[1] The design of the box is based upon that of Keeble and Gamble.

The counts were made by dividing the field into three areas, namely, the illuminated, the mid-area and the dark. Owing to the fact, however, that the illumination in the mid-area must have been almost imperceptible, for practical results it might have been quite safe to include the mid-area counts with those of the dark area, but for sake of surety they have been considered as a separate area. In the greater number of cases it was an easy matter to count the number of individuals in each of the three areas before a change in position took place. The number of twenty individuals was, in most cases, used for experimentation, for so small a number distributed over three areas could be taken in at a glance, and furthermore twenty seemed a sufficient number to give representative results. In the following account are recorded experiments carried on with the first five stages *Homarus*. All the conditions of light and backgrounds were not brought to bear upon all five stages, and only a sufficient number of experimentation reports are here recorded to show the general drift of the results.

EXPERIMENT I.

Conditions: black background; sunlight dull; 20 first stage larvæ.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	2	6	12	
2	3	4	13	Reversed.
3	1	5	14	
4	3	4	13	Reversed.

Similar results were obtained when a greater intensity of light was used.

EXPERIMENT II.

Conditions: white background; sunlight dull; 20 individuals used, first stage.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	2	5	13	
2	2	2	16	Reversed.
3	2	3	15	Reversed.
4	1	5	14	

EXPERIMENT III.

Conditions: white background; sunlight bright; 30 individuals used, first stage.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	22	4	4	
2	18	7	5	Reversed.
3	20	7	3	

These results seem to show that the first larval stage of *Homarus* is negatively phototropic on a black background, with both dull and bright light; but that while on white backgrounds he is, under low intensity, negatively phototropic, if the intensity of light becomes greater he becomes positively phototropic. Similar results were obtained with second and third stage larvæ under similar conditions of light and background.

EXPERIMENT IV.

Conditions: white background; red monochromatic light; 20 first stage larvæ.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	2	1	17	
2	2	3	15	
3	3	4	13	Reversed.
4	6	2	12	

EXPERIMENT V.

Conditions: white background; blue monochromatic light; 20 first stage larvæ.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	12	4	4	
2	11	3	6	Reversed.
3	13	2	5	

These two experiments indicate that in the case of a white background and red monochromatic light, the first stage lobsters are negatively phototropic, while in the case of a white background and a blue monochromatic light the same lobsters are positively phototropic. This was naturally somewhat unexpected, but in all the experiments involving similar conditions of light and background, the second and third stage lobsters respond in a similar manner. In case, however, a black background is used with lobsters of the first three stages, numerous experiments demonstrate a negative phototropism under the conditions of both red and blue light.

EXPERIMENT VI.

Conditions: black background; 20 early fourth stage lobsters; sunlight bright.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	13	3	4	
2	15	3	2	Reversed.
3	12	4	4	
4	15	4	1	

When experiments were tried with the fourth stage, however, a different reaction was found to occur. On black backgrounds and with lights of any intensity or color, the fourth stage lobsters appeared, contrary to the first three stages, positively phototropic, as the following table demonstrates. The degree of light intensity made no further change in the results, save that in instances where the light was the least intense the reaction was least marked; and when the light was most intense, as obtained by reflecting rays of light by means of a mirror into the tube, the definiteness of reaction was most evident.

When white backgrounds were used in connection with the fourth stage lobsters, it was found that in every case except with the monochromatic red light, a positive phototropic reaction resulted. The latter, which was also contrary to expectations, may be outlined as follows:

EXPERIMENT VII.

Conditions: white background; monochromatic red light; 20 early fourth stage lobsters.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	8	5	6	
2	1	6	13	Reversed.
3	1	10	9	Reversed.
4	4	5	11	

These resulting reactions in the case of the early fourth stage lobster may offer an explanation for the fact that this stage is so frequently caught in tow-nets drawn over the surface of any of our shore waters, while it has been a very unusual occurrence to secure in this manner either the earlier or the later stages. The same causes may also have worth for the reported facts that certain stages of the free-swimming larvæ of other forms of crustacea are found more frequently at the surface than are other larval stages of the same species.

It was a noteworthy fact, however, that old fourth stage lobsters would never manifest positive phototropic reactions with the same degree of certainty as that demonstrated in the case of younger fourth stage lobsters. Indeed, in a number of instances, fourth stage individuals which were due to molt within a period of one or two days, manifested on black backgrounds a definite tendency towards a negative phototropic reaction. This response was assumed without exception after the lobsters had molted into the fifth stage, and this reaction was manifested with any combination of light intensity, color or background. The following tables show the reactions in the case of the late fourth and the fifth stage lobsters.

EXPERIMENT VIII.

Conditions: black background (similar results were seldom obtained on a white background); sunlight bright; 20 late fourth stage lobsters.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	9	4	7	
2	11	5	4	Reversed.
3	7	6	7	
4	9	3	8	

EXPERIMENT IX.

Conditions: black background; medium sunlight; 12 fifth stage lobsters.

Test.	Lt. End.	Mid-area.	Dk. End.	Cover.
1	2	4	6	
2	2	3	7	
3	2	2	8	Reversed.
4	1	3	8	

The results of these experiments may also explain, to a certain degree, the facts which appear through the observation of large numbers of the larval stages of *Homarus* when confined and exposed to different light conditions, as they may also interpret to some extent the behavior observed in the larval and early adolescent stages of lobsters under natural conditions of environment. The first three larval stages, when confined in the large twelve-foot white canvas bags in which they were observed, manifested at all times a marked tendency to sink toward the bottom—except perchance at night, when more active swimming is observed in all the stages. This tendency during the daytime could not be controlled in any way. At night, however, it was possible to evoke a seemingly positive phototropic reaction from any of the thousands of young larvæ in the large canvas bags. This was accomplished by means of an acetylene light so directed against a certain area of the white field of canvas that large numbers would at once group themselves thickly about the illuminated area, manifesting, in the case of the third and fourth stages, such an effort to come into the light area that they would often throw themselves partially out of water, causing thereby numerous surface ripples. Since, however, similar results could be obtained when a black background was employed with the acetylene rays, and since the results were not so definite when the incident rays struck the water perpendicularly as when they were thrown at an angle, it was assumed that these reactions were not true phototropisms, but were largely due to the effort on the part of the young lobsters to move in the direction of the incident light rays. This phenomenon was better observable in the fourth stage of *Homarus*, when the very definite rheotropic proclivity, first clearly observable in this stage, could be entirely broken up by introducing the incident rays either at right-angles to or in opposition to the direction of the current. The fourth stage lobsters, however, even under the natural conditions of light, swim actively at the surface. It is not until the fifth stage that the bottom-seeking and 'hiding-habit' is fully established.

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October 10, 1905.

THE REGENERATION OF LOST PARTS IN THE LOBSTER.

BY V. E. EMMEL.

Thirty-fifth Annual Report of the Commissioners of Inland Fisheries
of Rhode Island, made to the General Assembly at its
January Session, 1905, p. 81.

THE REGENERATION OF LOST PARTS IN THE LOBSTER.

1904.

PRELIMINARY REPORT

BY

VICTOR E. EMMEL,

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THE REGENERATION OF LOST PARTS IN THE LOBSTER.

PRELIMINARY REPORT

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INTRODUCTION.

Anyone who is familiar with the habits of the crab and lobster is acquainted with the remarkable fact that, if one seizes a lobster's claw and holds it fast or pinches it, the lobster is likely immediately to drop his whole limb, or chela, and scuttle away; thus he defends himself by leaving his weapon in your hand—a means of defence which, as some one has said, would be useful to pick-pockets.

But still more remarkable than the power of "casting a claw," or autotomy, is the power to reproduce or regenerate a new limb to replace the one which has been lost. After the lobster has thrown off its leg, a bud begins to grow on the remaining stump, and in a comparatively short time a complete appendage is developed. The power of thus regenerating parts of the body which have been lost is possessed by a large number of crustacean forms, but is most marked in the crab and lobster.

This power of autotomy and regeneration has become a question of considerable scientific interest. Not only so, but as the result of recent rapid perfection and growing importance of lobster culture as an industry, the subject is also becoming one of economic interest. Since lobsters attack each other viciously in the lobster cars and ponds, the keeping of lobsters as well as the packing and transportation is often attended with considerable loss. An examination of freshly taken lobsters will often show that as high as from 7 per cent. to 25 per cent. have lost one or both chelipeds, or big claws—a matter of some importance when we consider the choice quality of the cheliped for market purposes and their large proportion of weight.

The recent success in the hatching and rearing of lobster fry and the new possibilities which are thus developing in the lobster industry are adding new interest to every phase of the lobster's life and growth. From the standpoint of lobster culture there are advantages in making the intervals between the moulting periods as short as possible. The frequency of moulting depends upon a variety of conditions, both physiological and environmental. Warm weather, a more vigorous life and appetite, seem materially to hasten the moulting stage. In view of these facts, such questions naturally arise as what relations are there between the loss and regeneration of appendages and the process of moulting.

Within the last few years the subject of regeneration has been receiving a great deal of attention and scientific study, but it has not been completely worked out in the lobster—*Homarus americanus*. The Rhode Island Fish Commission in its work of solving the problem of lobster culture and the consequent study of the entire life-history and habits of the lobster has directed some of its attention to the phenomenon of regeneration, in regard to which the present paper is in the nature of a preliminary report.

HISTORICAL SKETCH.

Experimenters and Observations.—The subject of regeneration did not attract general attention until about the middle of the seventeenth century. At that time the remarkable observations and experiments of such men as Trembley, 1740; and Reamur, 1742; Bonnet, 1745; and Spallanzani, 1768, became known. They found that if a hydra was cut into three or four pieces, each piece would grow out again and form a new and perfect animal. Bonnet found that certain kinds of fresh water worms could be cut into as many as fourteen pieces and still each piece would reproduce a new worm having a new head and tail. Trembley spilt a hydra's head and obtained a double-headed hydra. Indeed, he succeeded by this way in getting even an eight-headed hydra. Spallanzani discovered that a tadpole could reproduce a new tail; that salamanders could renew both tail and legs; that slugs could regenerate a new head. The celebrated experiments of these naturalists aroused a widespread interest in the subject of regeneration, which at the present day is growing with increasing momentum.

Numerous investigators have entered this promising field and are expanding its boundaries in many directions. While a review of their experiments and results on a great variety of forms in both animal and plant life would be irrelevant to our present purpose, perhaps a brief sketch of the work which has been done in the crustacean group and some of the theories of regeneration will be of value in indicating a few of the lines of interest in our present problem.

Among the investigators who have studied the regeneration of lost appendages in crustacea are Reamur, 1712; Goodsir, 1844; Chantran, 1873; Brooks, 1873; Herrick, 1895; Herbst, 1896–1901; Miss M. I. Steele, 1904; and Morgan, 1898–1905.

Reamur began his experiments on crabs and lobsters, but the sea broke over and carried away his boxes or filled them with sand. He then experimented with crayfish. The following description in his own words is one of the earliest accounts of experiments with crayfish: "I took several of them from which I broke off a leg; I placed them in one of the covered boats which the fishermen call 'Bontiques,' in which they keep fish alive. As I did not allow them any food I had reason to suppose that a reproduction would occur in them like that which I had attempted to prove. My expectations were in not vain. At the end of some months I saw, and this without surprise, since I had expected it—I saw, I say, new limbs which took the place of the old ones which I had removed. They had the same form in all their parts, the same joints, the same movements.

A kind of regeneration like this hardly less excites our envy than our imagination; if, in place of a lost leg or arm, another would grow out again, one would be more willing to adopt the profession of the soldier."*

Reamur also noticed that the time necessary for the reproduction of new limbs varied with a number of conditions, such as the seasons and the temperature of the water—regeneration being more rapid in warm than in cold water; and legs broken off in winter did not grow out again until summer. He also cut off the tails of crayfish, but did not find that they were renewed.

Chantran, in the study of crayfish, made some observations upon the regeneration of the antennæ and eyes.

Herbst made his experiments upon decapods and isopods. He obtained some very interesting results in the regeneration of the eye. In place of an eye, he often found a new antenna-like organ. From his experiments he arrived at the conclusion that the presence or absence of the optic ganglion was the factor determining whether an eye or an antenna-like organ shall be reproduced; and if the optic ganglion is present the eye would be renewed, but if gone an antenna-like organ may be regenerated instead of an eye.

Miss Steele made a careful study of the regeneration of crayfish appendages. She succeeded in obtaining some interesting reproductions of antennæ-like appendages in place of the normal eyes.

Herrick, in his work on the American lobster, has given a concise account of his observations upon the regeneration of the chilipeds, antennæ, and some of the thoracic appendages. Brooks also studied regeneration in the European lobster—*Homarus vulgaris*. Some of the results of both Herrick and Brooks will be referred to again later.

Morgan experimented with the hermit crab to determine whether there was any relation between the power of regeneration and the liability to injury. His conclusion is that there exists no necessary causal connection between the two.

Such, in brief, is the nature of some of the observations which have been made on regeneration among crustaceans. A few words may be added to indicate some of the theories which have been proposed to explain the phenomenon of regeneration.

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*Herrick. Bulletin of U. S. Fish Commission 1895, p. 103.

THEORIES OF REGENERATION.

Reamur was among the first to suggest a philosophical explanation of regeneration. In the following words he expresses his belief that each limb must contain an infinite number of eggs or egg-germs: "We may suppose that these little limbs which seem to grow out were inclosed in a little egg and that when a limb was broken off the same juices which nourished this part were used to develop and bring to birth the little germs of a limb inclosed in this egg.*

Goodsir assumed the existence of glandular-like bodies at the base of the limbs, which supply the germ of the new limbs; but the existence of such glandular bodies is not supported by the results of other observers.

Pfluger (1883) assumed that food material is taken up at the wounded surface and organized into the substance of the new limb.

Herbert Spencer elaborated a comparison of regeneration with the process of restoring a broken crystal. He suggests that "analogous forces" are at work both in the renewal of a part of a crystal and in the regeneration of a limb.

Two opposing views exist at the present time as to the origin of the power of regeneration, of which Weismann is a representative of one and Morgan of the other. Weismann concluded that the regeneration power is a characteristic which has been acquired by natural selection. He found an important reason for this conclusion in a supposed causal relation between the power of regeneration and the liability to injury. Morgan, on the other hand, denies the existence of any such causal relations and concludes that the power of regeneration is not the result of any selective agency of the environment, but that "regeneration is a fundamental attribute of living beings"†

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*Herrick. U. S Fish Commission Report 1895. p. 106

†Morgan. Regeneration, p. 282.

At the present time there are also two fundamental and opposing views of the nature of the "organization of the organism," namely, those in which "organization" is explained as the result of the collective action of smaller units, and second, those in which the "organization" itself is regarded as a single unit which controls the parts.* The more recent hypothesis of regeneration may be roughly classified as coming under one or the other of these two interpretations of the organism.

The preformation theories of Bonnet and the germ theory of Weismann are representative of the first school. Weismann, with Bonnet, assumed the existence of latent germs in the different parts of the body. But Weismann went further, and located these germs in the nucleus of the cell. Regeneration, then, is caused by latent cells which contain pre-formed germs in the nucleus, called determinants.

Supporters of the other school, like Whitman and Morgan, strongly argue that the cell theory is too narrow a basis from which to interpret the organism, and that the organism has a structure of its own independent of that of the cells. It is maintained, for example, that such phenomenon as a certain "polarity," which is well illustrated in a piece of worm in which the anterior region always reproduces a head and the posterior end a tail—that such phenomena must be considered in an explanation of regeneration. At present a variety of experiments are being made to determine in what way the new regenerating material is "regulated" by the structure of the piece or of the organism.

Thus it may be seen that the question of regeneration is still an open one, and that what is needed is a larger body of careful and exact experiments and observations before we can hope for a final solution of the problem.

EXPERIMENTS AND OBSERVATIONS ON REGENERATION.

Systematic observations were made and material collected during the summer of 1904 at the experiment station of the Rhode Island

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*Morgan. Regeneration, p. 277.

Commission of Inland Fisheries at Wickford, R. I. In the study of the problem several questions are immediately suggested, as, for example, the extent of the power of regeneration throughout the organism, the length of time required for the reproduction of a normal appendage, and the exact process in the development of a given structure.

At the beginning of the work several definite questions were taken and an effort made to get data for their solution. The lobsters used in the following experiments may be conveniently described in two groups.

Group I includes about fifty lobsters, varying from six to nine inches in length. They were obtained directly from the fish-traps, through the kindness of the Lewis Brothers, and placed in floating cars. Each lobster was tagged, weighed, measured, and the sex and general conditions were noted. They were mutilated in a variety of ways and daily observations and measurements made on the regenerating processes, moults, etc.

Group II embraces about a hundred very young lobsters ranging from the fourth to the ninth stages, *i. e.*, from one-half to about two inches in length. The young lobsters were taken from the hatching bags after they had moulted into the fourth stage so that the exact age and stage of each lobster was known. They were placed in floating cars which had been divided into small compartments by wire screening. Mutilations, measurements, and other observations were systematically recorded and material preserved for further histological study.

It was attempted to conduct the experiments under as normal conditions as possible. The lobsters were fed on fish and clams; an awning was placed over the experiment cars; the cars were also constructed so as to provide for a free circulation of the water, and every precaution was taken to keep the lobsters in a nearly natural environment. The experiments were also made at a favorable season of the year,—the latter part of July, through the months of August, September, and October, and part of November.

EXPERIMENTS.

In the discussion of the experiments and observations it has seemed most convenient to arrange the data under the following subjects:

I. The power of regeneration throughout the organism.
II. The relative power of regeneration at different levels in the thoracic appendages.
III. The attainment of the normal length of the appendages.
IV. The effect of repeated removal of an appendage.
V. Regeneration and the process of moulting.

I. The Extent to which the Power of Regeneration Extends Throughout the Organism.

Naturally in examining a lobster in search of regenerating structures we would look at those organs which are most liable to injury. Our examination would soon make it evident that among the parts most exposed to injury are such appendages as the antennæ and legs, and it is, indeed in these appendages that the regenerating structures are most commonly seen.

Chelipeds and Ambulatory Appendages.—In order to obtain a conception of the normal conditions under which regeneration usually occurs in these appendages, a few words will be necessary in regard to the process of autotomy.

The chelipeds (big claw) is divided into seven parts or segments. All these segments are united by flexible joints with the exception of the second and third basal segments (Plate XXII, Fig. 1), the basiopodite (3), and ischiopodite (2). In the case of the cheliped the basiopodite, and ischiopodite are fused together into one immovable piece, but there is still a distinct groove marking the two segments (Fig. I, 1.). It is always precisely at this groove that separation occurs when the limb is thrown off by autotomy. Fig. II shows the basiopodite (3) and the ischiopodite (2) separated by autotomy at this groove or breaking plane (1.) Upon examining the broken surface of the basiopodite it is seen that this breaking plane is obviously the most favorable region of the cheliped for autotomy. Not only is it one of the narrrowest cross sections of the limb, but the external groove is continued inwards by a double membraneous plate, which readily separates when the limb is voluntarily dropped off, leaving one membrane upon the leg and one on the remaining stump. Fig. II, 4, shows this membrane extending almost entirely over the basiopodite—it is perforated only at the center by an artery, the blood sinuses, and a large nerve. It is remarkable that the nerve and blood vessels may be thus suddenly snapped off leaving the stump as clean as though it were cut with a sharp knife.

In the case of the ambulatory appendages or walking legs, the second and third segments (Fig. III, is, bs.) are not fused as in the chelipeds, but the legs are always thrown off at the joint between these two segments (Fig. III, 1). Throughout the organism the power of autotomy has been observed to exist only in the chelipeds and the four pairs of walking legs.

When the limb has been thrown off a dense blood clot immediately forms over the broken surface of the stump. Within a few days a small white papilla appears near the center of this dot which soon develops into the bud of a new limb. The time which intervenes between autotomy and the appearance of the new bud varies with several conditions which will be referred to later, such as the age of the lobster and the moulting period, etc. Fig. IV, Plate XXII, shows the basiopodite of the first left leg of a 7⅛ inch lobster, on which the regenerating bud of three millimeters has appeared within about fifteen days after autotomy. In the same number of days a fourth or fifth stage lobster will often have reproduced an entire appendage.

The bud continues to increase in size, sometimes becoming more than an inch in length. Fig. I (1), Plate XXI, shows the regenerating bud of the left cheliped of a 7½ inch lobster which measured 1⅜ inches just before moulting. Throughout this process the bud is always inclosed within a membraneous cuticular sac. In the latter part of the development constrictions begin to appear upon the bud which mark the formation of the future joints and segments of the limb. The papilla and young bud are first white and then become pink in color. Later the color becomes darker, and just before moulting the whole bud usually presents a very dark bluish red or purple color. The appearance of this dark color is valuable to fishermen as an index of an approaching moult of the lobster.

Both chelipeds and the four pairs of walking legs regenerate from the breaking plane. The phenomenon of regeneration at different levels will be described later.

Antennæ.—The antennæ, as has already been noted, are very liable to injury, but they do not possess the power of autotomy. This may be due to the fact that even a part of the antennæ may still be of service to the lobster, and therefore a provision for its autotomy is unnecessary.

Both antennæ and antennules regenerate very readily at any level, either in the long flagellum or the articulations at the base. When the flagellum regenerates from its base the growing bud soon assumes a sickle shape and then coils upon itself in a spiral form. In some cases as many as five or six bright red colored spirals were observed just before a moult. When regeneration occurs in the segsegments of the flagellum a bud does not appear externally but the development takes place within the broken tip, and the new part of the antenna is only apparent after the next moult. Both methods of renewal seem to be an admirable provision for preventing an undue exposure of the delicate buds to injury.

The Eyes and Maxillepeds.—The maxillepeds, although not so liable to injury as the antennæ or limbs, seem to possess a comparatively normal power of regeneration.

In the experiments on the eyes the results were not conclusive. The eyes of nine lobsters were mutilated either by the removal of a part of the cornea or of the whole eye-stalk. The lobsters averaged about eight inches in length; the mutilations were made in the latter part of July and the first week in August, and in most cases soon after a moult, thus securing the most favorable conditions for restoration. Three of the lobsters were in a normal condition, that is, they had no other appendages which were regenerating; the other six had various appendages in the process of renewal. The observations were continued through the middle of October, but no positive evidence of regeneration was apparent.

In view of the fact that Herbst and Miss Steele have obtained such remarkable results in the regeneration of the eye in the crayfish and other decapods it may be expected that these results will be modified by further experiments, although I am not aware of any account of regeneration in the eye of the lobster. But while the present results may not be sufficient to justify a positive statement, it is certainly evident that the regeneration of the eye is comparatively very slow, especially when it is considered that in the same lobsters and at the same time other appendages were regenerating in a normal way.

Abdominal Appendages. The first pair of abdominal appendages are modified into external reproductive organs. Several observations were noted of their regeneration. In experiments with the other four pairs of abdominal appendages, or swimmerets, positive results were obtained in the second and third pairs, and it seems safe to say that all the swimmerets will regenerate. Fig. I, 2, Plate XXI, shows the regenerating bud of the second left swimmeret; in Fig. II is seen the same appendages after moulting. Several instances were also noted in which the swimmerets regenerated from different levels, as, for example, the restoration of the endopodite and exopodite when one or both were removed.

It may be of interest to note that the swimmerets do not seem to regenerate readily in the crayfish, at least Miss Steele did not meet with success in obtaining their restoration. Morgan, from his observations with the crab, suggests that the comparative weakness in the power of regenerating the swimmerets may be partly due to a smaller supply of food material as compared with the thoracic appendages.* This interpretation will be referred to again.

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*Morgan. Zoölogical Bulletin, Vol. I, No. 6, p. 299.

Other Parts of the Organism.—The beak will be renewed when broken off. Regeneration was observed in various segments of the telson. That the carapace possesses some power of regeneration seems to be indicated by the fact that around holes made in the dorsal region of the thorax the exoskeleton was partially restored after a moult. Some of the gills were removed from several lobsters, but no regeneration was noted during the period of observation.

It is thus seen that the ability to regenerate lost parts is remarkably extensive throughout the external structure and appendages. No definite observations have yet been made on the power of regeneration in the internal organs. Future experiments in this direction may furnish interesting data, especially in the case of internal organs not exposed to injury.

II. The Relative Power of Regeneration at Different Levels in Thoracic Appendages.

As has just been stated, the walking legs are always thrown off by autotomy at the breaking plane between the second and third segments. Now when it is considered that the regeneration of the leg usually occurs at this joint the question is immediately suggested, *will the thoracic appendages regenerate at other levels either distal or proximal to the breaking plane?*

For the purpose of answering this question the legs were cut at the various levels indicated in Figs. III, V, Plate XXII. As shown in Fig. V, the segment or basiopodite just below or proximal to the breaking plane was cut at three different levels: pa indicates a cut at the joint between the basiopodite and coxopodite; pb, a plane through the middle of the basiopodite; and pc, a level just below the surface of the breaking plane. Fifteen mutilations were made below the breaking plane of the chelipeds and walking legs; seven limbs were cut at pa, five at pb, and three at pc. Out of the fifteen mutilations only three showed any regeneration, and those were at the level pb or from the middle of the basal segment.

Thirty-nine mutilations were also made at regions distal to the "breaking plane," and at the levels 1db., 2db., and the joint between the fourth and fifth segments. (See Fig. III, Plate XXII, 1db, 2db, 3.) Eleven regenerating structures were obtained, seven from the level 1db and four from the joint. Plate XXII, Fig. VI., shows the second right leg regenerated from the level 1db, or the middle of the propodite. This claw and half segment were restored at the next moult, which occurred two months and eleven days after mutilation. It will also be noted that the renewed structure is proportionately much smaller than the normal size.

The above data may be tabulated as follows:

TABLE I.

	Mutilation levels.	Number of Limbs Mutilated.				Total number mutilated.	Total number regener.
		Cheliped.	1st leg.	2nd leg.	3rd leg.		
Below breaking plane.	pa	2	5	....	....	7	0
	pb	....	4	....	....	5	3
	pc	1	....	....	2	3	0
Above breaking plane.	1db	....	2	7	7	16	7
	2db	....	1	4	2	7	0
	3rd joint.	....	....	8	8	16	4

From the table it may be seen that one-fifth of the appendages were restored from levels below or inside the breaking plane and nearly one-third were renewed at levels distal to the same plane.

These results show that the thoracic appendages may regenerate from levels either distal or proximal to the breaking plane. The comparatively larger per cent. of restorations from the distal levels also indicate that the regenerating power varies at different regions in the limb.

While the experiments just described were in progress observations were also made on *the power of regeneration at different levels as compared with the same process at the breaking plane.*

In the above experiments the limbs of twenty-eight lobsters were mutilated. The mutilations were all made at about the same time, viz., the first of August. For the purpose of comparison the limbs were not only cut at the levels noted above, but in nearly every case some of the other legs were at the same time autotomously removed at the breaking plane. In the few exceptional cases some of the legs were already gone, so removal was unnecessary. During the three months of observation all but seven of the lobsters moulted. In all these lobsters the limbs which had been broken off at the breaking plane began to regenerate in the usual manner, while of the appendages mutilated proximally or distally of this plane, only a small per cent. showed any sign of regeneration; or, more exactly, each of these twenty-eight lobsters had limbs gone at both the breaking plane and parts which were cut at other levels; nearly one hundred per cent. of the lobsters showed regeneration at the breaking plane and only twenty-five per cent. showed a similar process at the other levels.

The following tabulated data on four of these lobsters is characteristic of the results obtained:

Data on Four of the Twenty-eight Lobsters used in the Experiments on Regeneration at Different Levels.

Lobster.	Appendages.	Mutilations.	Date.	Length.	Date	Length.	Date.	Length.	Date.	Length.	Date.	Length.	Length after moult.
No. 34.	1st R. leg...	*Aut. Aug. 1...	Aug. 19..	1.5 mm..	Aug. 27..	4.5 mm..	Sept. 5...	11.0 mm.	Sept. 17..	18.5 mm.	....	....	56.0 mm.
Length, $7\frac{13}{16}$ in.	1st L. leg...	†Cut pa, Aug. 4	Aug. 19...	0.0 mm..	Aug. 27..	0.0 mm..	Sept. 5...	0.0 mm.	Sept. 17..	0.0 mm.	....	....	0.0 mm.
Male.	2nd R. leg..	Aut. Aug. 1....	Aug 19..	3.0 mm..	Aug. 27..	9.5 mm..	Sept. 5...	15.5 mm.	Sept. 17..	20.0 mm.	....	....	56.0 mm.
Moult, Sept. 24.	2nd L. leg...	Aut. Aug. 1....	Aug. 19..	1.5 mm..	Aug. 27..	6.0 mm..	Sept. 5...	14.0 mm.	Sept. 17..	22.0 mm.	....	....	56.0 mm.
No. 28.	R. cheliped..	Aut. Aug. 1....	Aug 19..	pap......	Aug. 27..	3.0 mm..	Sept. 5...	9.5 mm.	Sept. 17..	23.5 mm.	Sept. 27..	32.0 mm.	81.0 mm.
Length, $6\frac{8}{16}$ in.	L. cheliped..	Cut pa, Aug. 2..	Aug. 19..	0.0......	Aug. 27..	0.0 mm..	Sept. 5...	0.0 mm.	Sept. 17..	0.0 mm.	Sept. 27..	0.0 mm.	0.0 mm.
Female	2nd R. leg..	Aut. Aug. 1....	Aug. 19..	2.5 mm..	Aug. 27..	6.0 mm..	Sept. 5...	12.5 mm.	Sept. 17..	20.0 mm.	Sept. 27..	25.0 mm.	56.0 mm.
Moult, Sept. 28.	2nd L. leg...	Aut. Aug. 1....	Aug. 19..	3.0 mm..	Ang. 27..	7.5 mm..	Sept. 5...	14.0 mm.	Sept. 17..	22.0 mm.	Sept. 27..	23.5 mm.	58.0 mm.
No. 30	R. cheliped..	Aut. Aug. 1....	Aug. 19..	1.5 mm..	Aug. 27..	4.0 mm..	Sept. 5...	8.0 mm.	Sept. 17..	15.5 mm.	Sept. 27..	26.5 mm.	87.5 mm.
Length, $7\frac{15}{16}$ in.	L. cheliped..	Cut pa, Aug. 2..	Aug. 19..	0	Aug. 27..	0.0 mm..	Sept. 5...	0.0 mm.	Sept. 17..	0.0 mm.	Sept. 27..	0.0 mm.	0.0 mm.
Male.	2nd R. leg..	Aut. Aug. 1....	Aug. 19..	pap......	Aug. 27..	2.0 mm..	Sept. 5...	6.0 mm.	Sept. 17..	12.0 mm.	Sept. 27..	22.0 mm.	84.5 mm.
Moult Oct. 28.	2nd R. leg..	Aut. Aug. 1....	Aug. 19..	pap......	Aug. 27..	3.0 mm	Sept. 5...	5.5 mm.	Sept. 17..	12.5 mm.	Sept. 27..	20.0 mm.	83.0 mm.
No. 100.	2nd R. leg..	Gone, July 25..	Aug. 14..	4.5 mm..	Aug. 20..	9.5 mm..	Sept. 1...	12.5 mm.	Sept. 10..	15.5 mm.	....	....	60.0 mm.
Length, 7 in.	3rd R. leg....	Gone, July 25..	Aug. 14..	15. mm..	Aug. 20..	4.5 mm..	Sept. 1...	11.0 mm.	Sept. 10..	14.0 mm.	....	....	62.5 mm.
Female.	1st R. swim.	Cut July 25....	Aug. 14..	0........	Aug. 20..	pap.	Sept 1...	1.5 mm.	Sept. 10..	1.5 mm.	....	....	17.0 mm.
Moult, Oct. 10.	1st L. leg...	Cut pb, July 25.	Aug. 14..	0........	Aug. 20..	0.0 mm..	Sept. 1...	0.0 mm.	Sept. 10..	0.0 mm.	....	....	0.0 mm.

i. e. removed at the breaking plane. †Removed below breaking plane.

This table illustrates the fact that in the same lobster in which no regeneration occurred below the breaking plane regeneration did take place at this plane at a normal rate. It is evident from these results that while the thoracic appendages may and often do regenerate from other levels, the process is much slower in starting and there is a large difference in the frequency of restoration as compared with regeneration at the breaking plane; in other words, the tendency to regenerate a lost structure is much stronger and more highly developed at the normal breaking plane than at any other level in the limb.

In attempting to account for these facts the question is suggested whether the difference in regenerative power at different levels may not be best explained by the liability to injury and the action of natural selection. As has already been intimated, it is a matter of controversy whether the law of natural selection furnishes a satisfactory explanation of the power of regeneration. A disputed question in this controversy is whether there is any causal relation between the liability to injury and the capacity for regeneration. Weismann, for example, holds that there is such a causal relation, while Morgan, on the contrary, maintains that no such relation necessarily exists and that consequently natural selection is inadequate to explain the phenomenon of regeneration.

Now it seems unquestionable that in the thoracic appendages the breaking plane is the region of the limb most liable to final injury, *i. e.*, although the *initial* injury may be near the extremity, the *final separation* of the limb from the body usually *occurs at the breaking plane.* In all the lobsters taken from the traps during the summer the lost limbs were nearly always separated at that place—a leg or cheliped with half or two-thirds of the original number of segments remaining is seldom found. Yet this condition might naturally be expected.

In experiments upon autotomy in the lobster it was found that if the cheliped or leg was crushed by a pair of tweezers it was almost invariably dropped at the breaking plane. This was especially marked in young lobsters—the leg would be "shot off" almost simultaneously at the moment of injury. Great difficulty was experienced with the fourth and fifth stage lobsters because the stumps were almost always cast off at the breaking plane, no matter at what level the cut had been made. By the exercise of considerable care, however, the legs could usually be cut off, with a sharp instrument, near the tip without a loss of the total limb through autotomy. But if the cut was made near the base of the appendages, say in the region of the ischiopodite or meropodite, the remaining stumps were frequently dropped.

In all these experiments it is to be observed that a crushing injury resulted in the autotomy of the whole limb, and that success in preventing autotomy was only obtained by a quick cut of the instrument. This suggests a clue for the explanation of the fact that under natural conditions the lobster's limb is usually gone at the breaking plane.

Even a superficial examination of the construction of the lobster's claws will show that they are adapted for holding and crushing. Their effectiveness in removing an opponent's limb may be seen at any time in the lobster cars; indeed, their ferocious combats so often result in the loss of appendages that it is necessary to tie or "plug" the claws. It is perhaps safe to assume that the injuries sustained in other phases of the lobster's struggle for existence are of a similar character; that the lobster in the conflicts with his enemies more often suffers a crushing, tearing injury than a clean-cut amputation, and this results in the autotomy of the whole limb. This assumption is farther justified by the fact that the animals with which the lobster would most naturally contend are much better equipped for crushing and holding than for cutting. Thus both experiment and observation indicate that the breaking plane is the region of the lobster's limb at which it is usually severed from the body as the result of an injury to the limb.

It would seem, therefore, that the *power of regeneration is most highly developed at the breaking plane, i. e., the region at which the limb* *is usually severed from the body* as the result of an injury of the appendage. This leads to the conclusion that the high perfection of the power of regeneration at the breaking plane as compared with other levels, either distal or proximal to it, is causally connected with the liability to injury and, consequently, upon the basis of evolutionary principles, is the result of natural selection.

In this connection another observation may be cited, viz.: in some crustaceans, especially the crayfish and crab, and the same is also true in the lobster, the swimmerets are slower in restoration than the thoracic appendages. Some writers have suggested that the supply of food material is an important factor in producing this comparative difference; but the following experiment would indicate that such a factor should not be given too great importance in explaining this difference.

The results obtained in the following experiment illustrate one of a number of similar observations. In lobster No. 100, Table II, the first right leg and the first right swimmeret were removed on the same date, July 25th. The leg was cut at the middle of the basal segment or basiopodite, and the swimmeret was removed at its base. The next moult occurred two and one-half months after mutilation. The leg then showed no distinct regeneration, while the swimmeret was restored to about three-fourths the normal size. In such a case it is evident that, though the leg was more favorably situated than the swimmeret, with reference to the source of food material, its regeneration was less rapid. In passing it might also be observed that if the leg in this experiment had been removed a quarter of an inch farther out, *i. e.*, at the breaking plane, it would have regenerated very rapidly (see page 96), although in that case the supply of food material would, if anything, be even less. In conclusion, then, it may be said that, so far as the present observations go, the results indicate that some factor or factors, other than the supply of food material, determine the difference between the power of regeneration of the swimmerets and that of the thoracic appendages.

III. The Attainment of Normal Length in the Appendages.

Under this subject we will first consider *whether the thoracic appendages or legs will attain normal length at the first moult.*

Some investigators have maintained that the walking legs do attain the normal length at the first moult. The well-known zoölogist, Brooks, for example, made the following observations on the walking legs in a study of the European lobster, *Homarus vulgaris:* "After the ecdysis (moult) the new ambulatory limbs assumed all the proportions of their uninjured fellows, * * * this evidence shows that in the lobster, at any rate, the new ambulatory limbs attain their full development at the first ecdysis." He further proceeds to interpret this fact by remarking that, indeed, this is what we might be led to expect for the following reasons: A study of the functions of the different limbs shows that the chelipeds may still be of great service, even though they have not attained the normal length; but that it is different in the case of the walking appendages—a walking leg would be of little use to the lobster if it were much smaller than its fellows, and therefore there is a natural provision for the restoration of a walking leg to the normal size at the first moult.*

In regard to this question my observations do not support these conclusions. On the contrary, in the present experiments on a large number of lobsters, the ambulatory appendages did not thus attain their normal length at the first moult, as may be seen in the following tabulated data on five of these lobsters:

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*Brooks. Notes on Reproduction of Lost Parts in the Lobster (*Homarus vulgaris*). Roy. Physical Soc., session CXVI., pp. 370-385; 1887.

TABLE III.

Number.	Date.	Stage.	Inches.	Appendage.	Condition.	Moult.	Length.
2	Aug. 17	7ht.	....	3rd R. leg	Normal	Sept. 3	10mm.
				3rd leg	Removed		8½mm.
3	Sept. 10	7th.	....	4th R. leg	Removed	Oct 2	10mm.
				4th L. leg	Normal		12mm.
6	Sept. 6	7th.	....	4th R. leg	Removed	Sept. 27	11mm.
				4th L. leg	Normal		13mm.
114	Aug. 15	....	6¾	2nd R. leg	Normal	Sept. 15	81mm.
				2nd L. leg	Found reg.		62mm.
108	Sept. 1	....	6⅜	4th R. leg	Found reg.	Sept. 9	62mm.
				4th L. leg	Found reg.		47mm.

It will be seen in this table that three of these five lobsters were very young and that the other two were more mature, *but in no instance had the regenerating limbs attained the normal size at the first moult.* Moreover, in the case of the last lobster, No. 108, it may be observed that both members of the second pair of legs were regenerating at the same time. But even then, at the next moult, the new pair of legs were not the same size when compared with each other and differed by as much as fifteen mms. in length.

These data are characteristic of a large number of similar observations, so the conclusion seems evident that the walking legs do not always attain the normal length at the first moult; it also follows, therefore, that the interpretation that the walking legs regenerate to a normal size at the first ecdysis for functional or other purposes is not necessarily true.

A word might be added with reference to Brooks's observations. It is quite probable that his results may be explained in another way. It should be observed that my experiments were made during the summer months. Now, if a limb were removed during the winter, in which case the next moult would not occur for a comparatively long time, it is possible that under such conditions the given limb would more nearly approach the normal size at the first moult. This suggests an explanation of Brooks's results. From an examination of his data it appears that the lobster which he cites had lost the ambulatory limbs in question during the winter (the exact date is not given); consequently the new leg had a comparatively long period to regenerate before the next ecdysis in the spring, and so attained more nearly the normal proportions at the first moult.

If the thoracic appendages then do not attain the normal length at the first moult the question which next arises is: *How long does it take a regenerating appendage to attain the normal size?* Of course this question must be answered in terms of moulting periods rather than in terms of days and months, because the rapidity of regeneration, like the frequency of moulting, varies with the age of the lobster.

There is a popular belief that a lobster's appendages will be restored to normal length in one or at least two moults. The present observations, however, do not seem to justify such an opinion. In all the data there was no exception to the following observations: neither the antennæ, maxillepeds, thoracic appendages, the first pair of abdominal appendages (accessory reproductive organs), nor the swimmerets attained normal length at the first moult. In regard to the second moult a difficulty was encountered through the fact that in the mature lobster only one moult usually occurred during the summer. In the few cases in which data were obtained on the chelipeds through the second moult, those limbs had not yet grown to normal length. Fig. II, Plate XXI, is a good illustration of the comparative size of the regenerated and normal chelipeds just after the first moult. Figs. I and II on the same plate also show the remarkable expansion which occurs in the regenerating structure as soon as it is released through the moulting process from the membraneous sac of the bud.

This difficulty, arising from a long interval between the moulting periods, disappears in case of experiments with very young lobsters. The right chelipeds of lobsters varying from the fourth to the ninth stages were removed and the regenerating structures compared with the normal ones. Twenty-four measurements of the chelipeds were taken. Sixteen measurements were made at the first moult, seven were continued through the second and one through the third moult. With one exception the chelipeds failed to obtain the normal length. In this one exceptional case the cheliped had grown to its normal size, but only after the third moult, as is shown by the following data:

DATA ON LOBSTER NO. 10.

Stage.	Appendage..	Mutilation...	Moult...	Length.	Moult...	Length.	Moult...	Length.
(5)*	R. cheliped..	Removed....	Aug. 5..	10 mm..	Aug. 19.	13½ mm.	Aug. 27.	18 mm.
....	L. cheliped:	July 25......	(6)	12 mm..	(7)	15 mm..	(8)	18 mm.

*Moulted to fifth stage July 24.

Although in this instance the right cheliped did attain normal length in three moults, it is not certain that the limbs will always generate to the normal size in that time.

Thus it appears that no definite statement can be made as to the time required for an appendage to be restored to its normal size.

IV. EFFECT UPON THE REPEATED REMOVAL OF THE SAME APPENDAGE.

Curiosity might raise the question: How often could a lobster restore such a complex mechanism as a cheliped, or, in other words, what would be the effect of the repeated removal of a given limb? But the question also draws some interest from theoretical considerations. In a previous sketch of the theories of regeneration attention was called to Reamur's "egg-germs" and Weismann's "determinants." In considering such theories the query might naturally arise whether it would be possible to exhaust the "egg-germs," "determinants," or whatever the latent forces may be which contain the power of reconstructing the mechanism of the limb.

As a matter more of curiosity than of deliberate expectation that such an exhaustion of the regenerative power would be possible, a series of observations were made on young lobsters. Young lobsters were taken because in them the appendages are regenerated much more quickly and thus offer a larger number of opportunities to remove the limbs during the period in which the present observations were being made. The experiments were made upon the right cheliped. The given cheliped was always removed when it had regenerated and come out of the next moult. After each moult both regenerated and normal limb were measured as carefully as possible so as to determine the proportional gain of the new cheliped as compared with the normal one. It was attempted to remove the given limb immediately after each moult, so as to always allow the whole period between two moults for its regeneration. The attempt was also made in every way to keep all the conditions as uniform as possible.

The following table gives data on six of these lobsters:

TABLE IV.

Number.	Date.	Stage.	Appendage.	Mutilation.	Moult.	Length.	Per cent. gain of regen.	
I.	Aug. 24...	6	R. cheliped..	Removed...	Sept. 6......	14 mm.	$77\frac{7}{10}$	I.
			L. cheliped..	....		18 mm.		
	Sept. 6...	7	R. cheliped..	Removed..	Sept. 28 (?)..	17 mm.	79	
			L. cheliped..	....		21½mm.		
	Sept. 29..	8	R. cheliped..	Removed..	Nov. 11 (?)..	15 mm.	$57\frac{6}{10}$	
			L. cheliped..	....		26 mm.		
II.	Aug. 25...	6	R. cheliped .	Removed..	Sept. 7......	11 mm.	$78\frac{5}{10}$	II.
			L. cheliped..	....		14 mm.		
	Sept. 7...	7	R. cheliped..	Removed..	Sept. 28 (?)..	13 mm.	$72\frac{2}{10}$	
			L. cheliped..	....		18 mm.		
III.	Aug. 24...	6	R. cheliped..	Removed..	Sept. 6......	17 mm.	$77\frac{2}{10}$	III.
			L. cheliped..	....		22 mm.		
	Sept. 6...	7	R. cheliped..	Removed..	Sept. 27 (?)..	19 mm.	$70\frac{8}{10}$	
			L. cheliped..	....		27 mm.		
IV.	Aug. 23...	6	R. cheliped..	Removed..	Sept. 3......	13 mm.	$79\frac{3}{10}$	IV.
			L. cheliped..	....		16½mm.		
	Sept. 3...	7	R. cheliped..	Removed..	Sept. 19.....	14 mm.	$73\frac{6}{10}$	
			L. cheliped..	....		19 mm.		
	Sept. 19..	8	R. cheliped. .	Removed..	Oct. 25......	17½mm.	$74\frac{4}{10}$	
			L. cheliped..	....		23½mm.		

TABLE IV.—Continued.

Number.	Date.	Stage.	Appendage.	Mutilation.	Moult.	Length.	Per cent. gain of regen.	
V.	Aug. 23...	6	R. cheliped..	Removed..	Sept. 6......	15½mm.	90	V.
			L. cheliped..	....		17mm.		
	Sept. 6...	7	R. cheliped..	Removed..	Sept. 28 (?)..	17 mm.		
			L. cheliped..	....		23 mm.	73$\frac{9}{10}$	
VI.	Aug. 17...	6	R. cheliped..	Removed..	Aug. 27......	0 mm.	0	VI.
			L. cheliped..	....		13½mm.		
	Aug. 29...	7	R. cheliped..	Removed..	Sept. 8 (?)...	13 mm.	83$\frac{8}{10}$	
			L. cheliped..	....		15½mm.		
	Sept. 10..	8	R. cheliped..	Removed..	Oct. 2 (?)....	15 mm.	75	
			L. cheliped..	....		20 mm.		

It will be seen that at each removal the right cheliped usually showed a diminishing per cent. of gain when compared with the corresponding normal appendage; in other words, in each successive regeneration there was a larger per cent. of difference between the regenerated and normal limb. Interesting results might, perhaps, be obtained by a continuation of such an experiment through a much longer period of time; but it would be hasty to conclude that the above data proved a decrease in the regenerative power, because, as will be noticed, each successively regenerated cheliped does show a per cent. of gain when compared among themselves; as, for example, in lobster No. 4 the measurements for the right cheliped at the successive moults are 13, 14, and 17½mm., thus showing a continuous increase (although the same is not true in lobster No. 1). So the present data are insufficient for a positive answer to the original question whether the regenerative power could be decreased by continuous mutilation, and the above results are given rather as a matter of incidental interest than as indicating a definite conclusion.

V. Regeneration and the Process of Moulting.

The moulting process of the lobster is in itself a most fascinating phenomenon. At certain more or less regular periods the lobster removes not only the chitinous shell of his body, but also the entire covering of all the appendages, eyes, and even the stomach, as a boy might remove his old clothes for a brand new suit. In considering this important as well as critical period in the lobster's life, the question is suggested —*what relations are there between the regeneration of the thoracic appendages and the process of moulting*. If, under such circumstances, a limb did begin to regenerate and had only become a small bud at the time of moulting, what would be its condition after the moult? Would it continue after the moult merely as a growing bud, or would it be a functional appendage, *i. e.*, have all the activities of a fully developed limb?

In the data collected on the relation of regeneration and the moulting process the following interesting fact was obtained: In all the observations made, not only upon the lobsters used in the experiments, but also upon the lobsters at the hatchery, *there was not one instance in which a lobster came out of the moult with an appendage which was in a non-functional condition.* In other words, in every case a regenerating appendage which continued through a moult had all the motions and uses of a perfect limb, *i. e.*, "it moulted functional." The conclusion is, therefore, that a regenerating limb which passes through an ecdysis always moults as a functional appendage.

If this conclusion is correct, then two alternatives seem to present themselves. Either a regenerating structure which has not developed far enough to moult "functional" will be dropped during the moult; or else the limb will only begin to regenerate when the interval between the time of injury and the date of the next moult is long enough to permit a degree of development such that the limb can moult with "functional" characteristics.

With regard to the first phase of the question it can be stated that no conclusive evidence was obtained that partially developed regenerating buds were dropped during a moult. In regard to the second alternative, that a limb will not begin to regenerate unless the interval between the time of injury and the date of moulting is sufficient to permit the development of a functional appendage, two questions arise. First, is there a definite time limit within which a limb will not begin to regenerate before a moult? Second, if a limb has begun to regenerate within a comparatively short time before the succeeding moult, will the rapidity of the regenerating process be materially hastened or the moulting date delayed so as to favor the restoration of the appendage?

Of these two questions we will consider first whether *there is a definite time limit within which a limb will not begin to regenerate before a moult.*

In a general way it may be said that there appears to be such a limit; for in a large number of instances it was found that an appendage would not regenerate if it had been removed shortly before a moult. Most of the data which furnished any definite evidence in regard to the comparative length of this limit was obtained from young lobsters. The following table shows the minimum number of days in which regeneration did begin, and the maximum number of days in which restoration did not begin, as noted in sixth and seventh stage lobsters:

TABLE V.

Number.	Date.	Stage.	Appendage.	Mutilation.	Moult.	Condition.	Time between mutilation and moult.
7	Aug. 23...	6	R. cheliped.. L. cheliped..	Removed..	Sept. 3.....	Reg...... Normal.	10 days.
12	Aug. 24...	6	R. cheliped.. L. cheliped..	Removed..	Sept. 6.....	Reg...... Normal.	13 days.
15	Aug. 24...	6	R. cheliped.. L. cheliped..	Removed..	Sept. 6.....	Reg...... Normal.	13 days.
11	Aug. 25...	6	R. cheliped.. L. cheliped..	Removed..	Sept. 9.....	Reg...... Normal.	14 days.
6	Aug. 23...	6	R. cheliped.. L. cheliped..	Removed..	Sept. 6.....	Reg...... Normal.	14 days.
14	Aug. 25...	6	R. cheliped.. L. cheliped..	Removed..	Aug. 27.....	Normal... no. reg..	2 days.
16	Aug. 26...	7	R. cheliped.. L. cheliped..	Removed..	Aug. 30.....	no. reg... Normal.	4 days.
18	Aug. 26...	7	R. cheliped.. L. cheliped..	Removed..	Aug. 30.....	No. reg... Normal.	4 days.

As far as these results go it appears that the maximum regenerating limit obtained was ten days and that the maximum non-regenerating internal was four days; or, in other words, the chelipeds of the sixth stage lobster will regenerate if removed ten days before the following moult, and that the same limbs will not regenerate if removed (two days in the case of the sixth stage or four days in the case of the seventh stage) before the next moult. No data has yet been secured to show whether a limb would or would not be restored if removed between four and ten days preceding a moult? In the more mature lobsters the shortest period noted in which a thoracic appendage began to regenerate was sixteen days.

These results, then, indicate that there is a certain period preceding an ecdysis in which if a thoracic appendage is removed, the limb will not begin to regenerate before the following moult. Whether this limit approaches any regular per cent. of the whole interval between two moults, it would require a larger number of comparative observations to determine.

The second question to be considered is *whether there is any adaptation of the regenerative or moulting processes favorable to the early restoration of a lost appendage.*

In the attempt to ascertain whether the moulting process is delayed, or, what is the same thing, whether the interval between moults is lengthened by the presence of regenerating structures, it is necessary to know first the average length of the moulting period of a normal lobster at a given stage. If this were known, then the question might be answered by observing whether that period is lengthened in a lobster with structures regenerating.

During the present summer Prof. A. D. Mead has collected data and made observations upon the moulting periods of young lobsters in which the stages are known. A tabulated form of Prof. Mead's results may be found in this report, page 40. This table shows that the average duration of the fifth stage for the normal lobsters was 9½ days; and of the sixth stage 12.7 days.

For these two periods, then, we have now a basis for comparison with the results obtained from experiments on mutilated lobsters. The following table gives the moulting periods of fourteen fourth, fifth, and sixth stage lobsters, in which one or more appendages were in the process of regeneration:

TABLE VI.

Group I.

No.	4TH STAGE.		5TH STAGE.		6TH STAGE.		7TH STAGE.	
	Date of Moult.	Stage Period.	Date of Moult.	Stage Period.	Date of Moult.	Stage Period.	Date of Moult.	Stage Period.
1			July 27	13 da.	Aug. 9	12 da.	Aug. 21	
2			July 27	13 da.	Aug. 9	14 da.	Aug. 23	
3			July 27	13 da.	Aug. 9	14 da.	Aug. 23	
4			July 24	16 da.	Aug. 9	18 da.	Aug. 27	
5			July 27	13 da.	Aug. 9	15 da.	Aug. 24	
6			July 26	14 da.	Aug. 9	28 da.	Sept. 6	
7			July 26	13 da.	Aug. 9	28 da.	Sept. 6	
8			July 27	13 da.	Aug. 9	18 da.	Aug. 27	
9			July 27	13 da.	Aug. 9	29 da.	Sept. 7	
10			July 27	13 da.	Aug. 7	13 da.	Aug. 20	
			Average 13.4 da.		Average 19 da.			

Group II.

11	July 12	15 da.	July 27	7 da.	Aug. 3			
12	July 12	15 da.	July 27	10 da.	Aug. 6			
13	July 12	16 da.	July 28	12 da.	Aug. 9			
14	July 12	19 da.	July 31	9 da.	Aug. 10			
	Average 16 da.		Average 9½ da.					

Group I in this table contains the moulting periods for the fifth and sixth stages of ten lobsters in which the right cheliped was removed immediately after each moult. It will be seen that the average length of the periods was 13.4 days for the fifth, and 19 days for the sixth stages.

A comparison of the length of the stages of these mutilated lobsters with the corresponding stages of the normal lobsters discloses the interesting fact that in the regenerating lobsters the length of the fifth stage was *forty per cent. and that of the sixth stage thirty-three per cent. greater* than the corresponding normal stages. This indicates that the *presence of regenerating structures tends to lengthen the interval between moults.*

Group II contains data on the moulting periods of four fourth and fifth stage lobsters. The right cheliped and second left leg of each lobster were removed about the middle of the fourth stage, but no appendages were removed in the fifth stage. The observations were continued through both stages. The point of interest is that in these specimens when the limbs had *not* been removed the length of the fifth stage period dropped back to the average of nine and one-haif days, the normal length for the fifth stage.

It will also be noticed that the average length of the fourth stage was sixteen days; since, however, the length of the normal stage has not yet been definitely determined, it cannot be stated how much longer this is than the normal fourth stage period.

In conclusion, these results indicate that the presence of regenerating structures tends to lengthen the interval between moults.

A series of observations were also made upon the first phase of our question, *i. e., whether the rapidity of the regenerating process is hastened by the approach of the moulting period.* This was done by removing some of the limbs of about twenty "chicken" lobsters and then making systematic observations and measurements on the regenerating structures. The given appendages were all removed at nearly the same time, about August first. Of course it was not known exactly when the lobsters would moult, but the observations were continued until they did moult and the results then compared to ascertain how the rate or regeneration varied with reference to the length of the interval intervening between the time of injury and the date of the moult.

The following data on four of these twenty lobsters show the nature of the results obtained on the chelipeds and second pair of ambulatory appendages:

TABLE VII.

Group I. Appendages removed About One Month and Twenty-five Days Before Moult.

GROUP I.	Appendages.	Date of Autotomy.	Date.	Reg.	Date.	Reg.	Date.
Lobster No. 34. Length $7\frac{3}{16}$ inches. Male.	R. cheliped..	Removed Aug. 1st.	Aug. 19.	3mm.	Aug. 27.	7½mm.	Sept. 5..
	L. cheliped..	Removed Aug. 1st.	Aug. 19.	3mm.	Aug. 27.	7mm.	Sept. 5..
	2 R. leg.....	Removed Aug. 1st.	Aug. 19.	3mm.	Aug. 27.	9mm.	Sept. 5..
	2 L. leg....	Removed Aug. 1st.	Aug. 19.	1.5mm.	Aug. 27.	6mm.	Sept. 5..
No. 82. Length $7\frac{3}{16}$ inches. Male.	R. cheliped..	Removed Aug. 1st.	Aug. 20.	pap.	Aug. 27.	4.5mm.	Sept. 7..
	L. cheliped..	Removed Aug. 1st.	Aug. 20.	....	Aug. 27.	3mm.	Sept. 7..

Group II. Appendages Removed Three Months Before Moult.

No. 30. Length $7\frac{5}{16}$ inches. Male.	R. cheliped..	Removed Aug 1st..	Aug. 19.	1½mm.	Aug. 27.	3½mm.	Sept. 5..
	2 R. leg....	Removed Aug. 1st.	Aug. 19.	pap.	Aug. 27.	3mm.	Sept. 5..
	2 L. leg.....	Removed Aug. 1st.	Aug. 19.	pap.	Aug. 27.	3mm.	Sept. 5..
No. 44 Length $7\frac{9}{16}$ inches. Male.	R. cheliped..	Removed Aug. 1st.	Aug. 19.	....	Aug. 27.	3mm.	Sept. 5..
	L. cheliped..	Removed Aug. 1st..	Aug. 19.	....	Aug. 27.	3mm.	Sept. 5..
	2 R. leg.....	Removed Aug. 1st.	Aug. 19.	pap.	Aug. 27.	3mm.	Sept. 5..
	2 L. leg......	Removed Aug. 1st.	Aug. 19.	pap.	Aug. 27.	3mm.	Sept. 5..

TABLE VII.—Continued.

Group I. Appendages Removed About One Month and Twenty-five Days Before Moult.—Continued.

Reg.	Date.	Reg.	Date.	Reg.	Date.	Reg.	Moult.	Length after Moult.
19½mm.	Sept. 17.	26½mm.	Sept. 24.	29½mm	....	....	Moulted Sept 26.	97mm.
18½mm.	Sept. 17.	29½mm.	Sept. 24.	31mm.	....	....		97mm.
15½mm.	Sept. 17.	20 mm.	Sept. 24.	22mm.	....	....		56mm.
15 mm.	Sept. 17.	21½mm.	Sept. 24.	22 mm.	....	....		53mm.
11½mm.	Sept. 17.	25 mm.	....	....	....	....	Moulted Sept. 28.	90mm.
11 mm.	Sept. 17.	23½mm.	....	....	....	....		100mm.

Group II. Appendages Removed Three Months Before Moult.—Continued.

8½mm.	Sept. 17 .	16½mm.	Sept. 27.	26½mm	....	....	Moulted Oct. 28.	97mm.
6 mm.	Sept. 17.	11½mm.	Sept. 27.	22 mm.	....	....		84mm
5½mm.	Sept. 17.	13 mm.	Sept. 27.	20 mm.	....	....		84mm.
7½mm.	Sept. 17.	18½mm.	....	....	Nov. 12.	26½mm.	Had not moulted Nov. 12.	....
8½mm.	Sept. 17.	17 mm.	....	....	Nov. 12.	26½mm.		....
9 mm.	Sept. 17.	17 mm.	....	....	Nov. 12.	23½mm.		....
7½mm.	Sept. 17.	17 mm.	....	....	Nov. 12.	23½mm.		....

It will be seen in this table that for the purpose of comparison the data have been arranged in two groups. In Group I, the next moult took place at about one month and twenty-five days after mutilation, while in Group II the lobsters moulted in the much longer period of three months after mutilation. The given appendages were all removed at nearly the same time. The lobsters were all males, and were about the average length of seven and three-quarters inches. They were all under the same conditions in regard to food, temperature, and season of the year. Thus all the conditions were evidently favorable for obtaining real comparative results.

An examination of the data shows that the appendages regenerated much more rapidly in the first than in the second group. This is clearly seen in a comparison of lobsters No. 34 and No. 30. The right cheliped of both lobsters were removed at the same date, August 1. Five of the measurements of the regenerated limbs, taken between the date of mutilation and the next moult, may be grouped as follows:

Lobster No. 34.	Aug. 19.	Aug. 27.	Sept. 5.	Sept. 17.	After moult. Sept. 26.
R. cheliped........	3 mm.	7½ mm.	19½ mm.	26½ mm.	97 mm.
Lobster No. 30.					Oct. 28.
R. cheliped........	1½ mm.	3½ mm.	8½ mm.	16½ mm.	97 mm.

In this comparison it is to be observed that in lobster No. 34 *in one month and twenty-five days the right cheliped not only regenerated twice as fast but it attained exactly the same length that the corresponding cheliped in Lobster No. 30 did in a period of nearly three months.* Similar comparisons might be made on all the appendages with the result that the limbs which were removed nearer the date of moult *regenerated more rapidly* than the limbs which were removed farther from the date of the moulting process.

It seems evident that there is an adaptation of the regenerative process to the moulting period, as seen in the fact that the growth of the regenerating structure may be materially hastened by the nearness of the approaching moult; and the interpretation seems to be that this adaptation is made so as to permit, as far as possible, the development of a *functional mechanism* before the moult. Thus a tentative answer is obtained for our original question.

In recapitulation, then, we may conclude from these observations that the following relations exist between the processes of moulting and regeneration: First, that a regenerating limb always moults as a functional appendage; second, that there is a definite time limit preceding a moult, within which a limb will not begin to regenerate; and third, that both regenerating and moulting processes maintain an adaptive relation to the regenerating limb, and thus furnish a doubly favorable condition for the development of a functional limb at the first moult.

If these conclusions are correct the question arises whether the moulting process or habit is not to be regarded as an important factor influencing the power of regeneration among crustacea—a question of especial interest when it is considered that one of the present features in the study of the phenomenon of regeneration in general is the determination of the external and internal factors influencing the process. The results from the present study of the relation between regeneration and the process of moulting, therefore, suggest the importance of further study of this subject.

SUMMARY.

The results and conclusions from the present series of experiments on regeneration in the lobster, *Homarus americanus*, may be summarized as follows:

I. Regeneration throughout the organism.

The power to regenerate lost parts is remarkably extensive throughout the external structure and appendages. The antennæ, antennules, maxillepeds, chelipeds, walking-legs, the first pair of abdominal appendages (accessory reproductive organs), swimmerets, telson, beak, and other parts of the carapace will be restored when injured.

II. The power of regeneration at different levels in the thoracic appendages.

1. The thoracic appendages have a varying power of regeneration at different levels throughout the limbs.

2. But this power is most highly developed at the "breaking plane," *i. e.*, at the joint between the second and third basal segments.

3. The fact that this "breaking plane" is also the region at which the limbs when injured are almost *always* autotomously severed from the body leads to the conclusion that the comparative perfection of the power of regeneration at this region may be explained as the result of natural selection.

4. In these experiments it was found that the swimmerets regenerated slower than the thoracic appendages; but it was also observed that the swimmerets will regenerate *sooner* than the legs if the latter were cut only a relatively short distance below the "breaking plane." So it may be questioned whether the supply of food material will explain the comparative difference in the restoration of the thoracic appendages and the swimmerets.

III. The attainment of normal length in the appendages.

The antennæ, maxillepeds, thoracic limbs, the first pair of abdominal appendages (accessory reproductive organs), and the swimmerets did not attain the normal length at the first moult.

The chelipeds of very young lobsters were observed through the first, second, and third moults with the result that the only case in which the cheliped attained a normal size was at the third moult.

IV. The effect of repeated removal of the same appendage.

The right cheliped of sixth, seventh, and eighth stage lobsters were repeatedly removed and regenerated. In the data obtained the given chelipeds showed a diminishing per cent. of gain when compared with the corresponding normal appendage, *i. e.*, in each successive regeneration there was a larger per cent. of difference between the lengths of the regenerated and the normal limbs.

V. Regeneration and the moulting process.

The following results were obtained from the observations on the relation between the regenerative and the moulting process:

1. A regenerating limb always moults as *a functional appendage.*

2. There is a definite limit preceding a moult within which a limb will not begin to regenerate.

3. There is an adaptation of both regenerative and moulting processes to the regenerating limb, favorable to the development of a functional appendage at the first moult after injury.

Plate XXI.—Shows regenerating appendages on the same lobster before and after moulting. (Photograph from life, $\frac{3}{7}$ natural size.)

Fig. I. Lobster just before moulting. Length, 7½ inches. Shows regenerating bud of second left swimmeret (2), and regenerating left cheliped (1).

Fig. II. Same lobster after moulting. Length, 8⅛ inches. Shows the regenerated left swimmeret (2), and left cheliped (1).

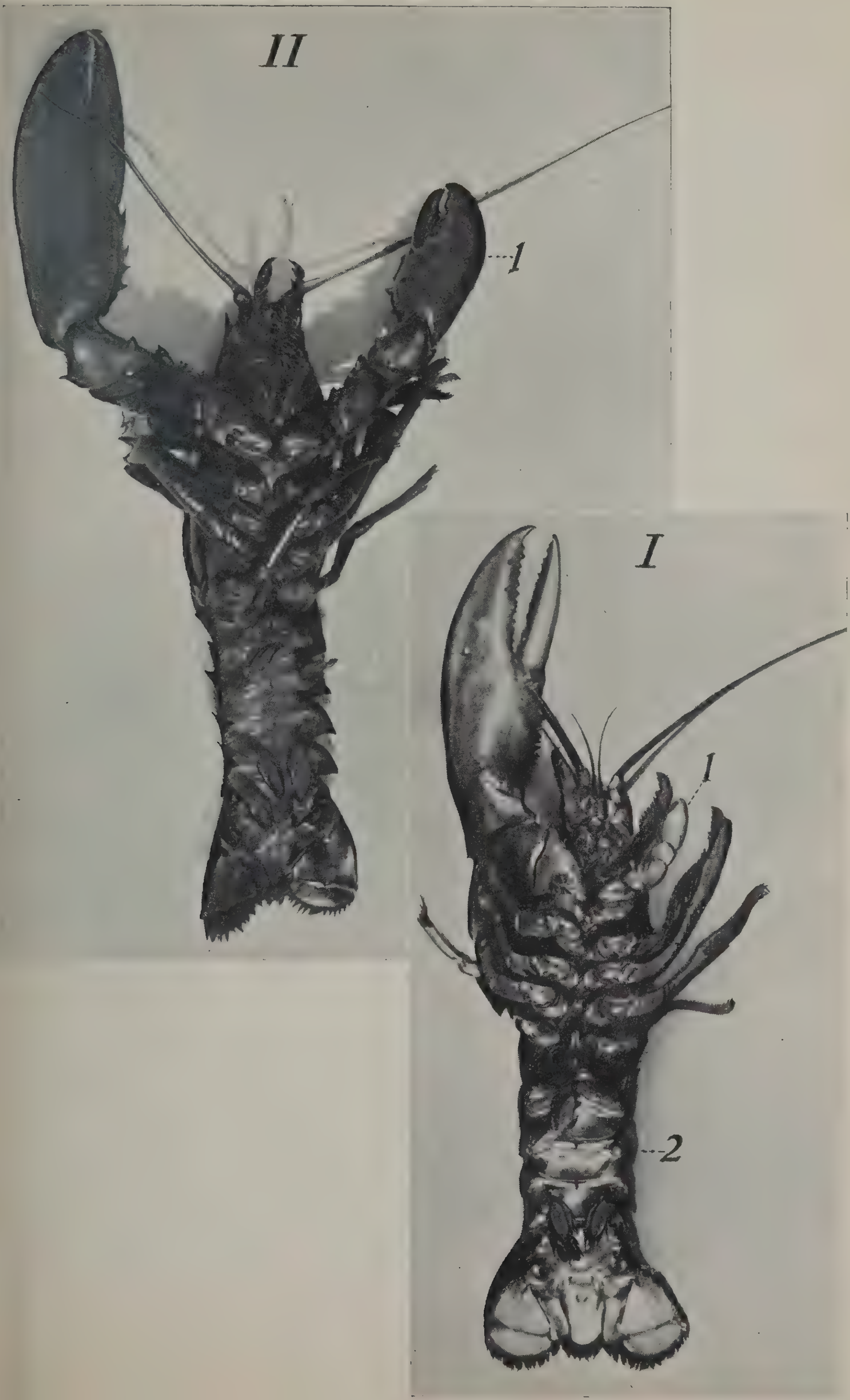

II
1
I
1
2

PLATE XXII.

Fig. I. Right cheliped before autotomy. The basiopodite (3) and ischiopodite (2) are seen united at the joint or breaking plane (1). Posteroventral view.

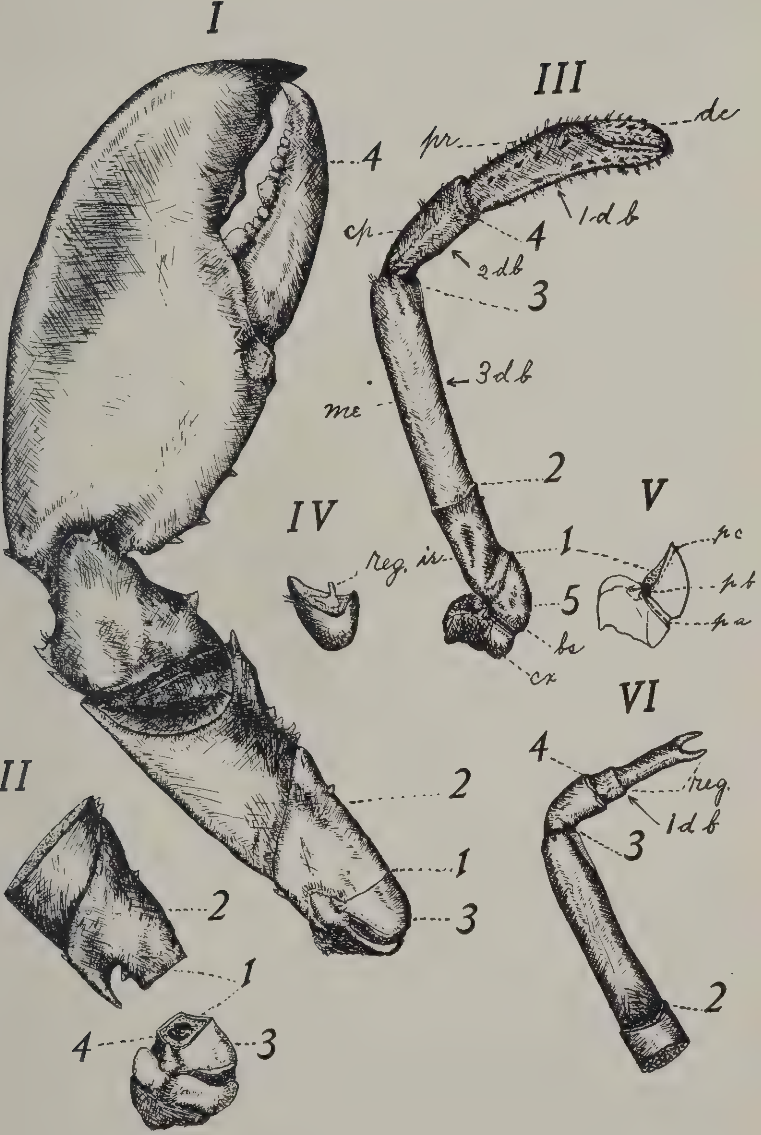
Fig. II. The same basiopodite (3) and ischiopodite (2) separated at the breaking plane (1) by autotomy. Over the broken surface of the basiopodite is seen the membraneous plate (4) of the breaking plane, perforated at the center by the blood vessels and nerve.

Fig. III. First right leg before autotomy. *cx*, coxopodite; *is*, ischiopodite; *me*, meropodite; *cp*, carpopodite; *pr*, propodite; *dc*. dactylopodite. The levels *1db*, *2db*, *3db*, and the joints 2, 3, and 4, indicate the different regions at which the limbs were cut in the experiments. Posterior view.

Fig. IV. Basiopodite of first left leg, showing 3 mm. regenerating bud, 15 days after autotomy.

Fig. V. Illustrates three levels at which the basiopodites were cut in the experiments: *pc*, just below the surface of the breaking plane; *pb*, thn middle of the segment; and *pa*, at the joint between the basiopodite and coxopodite.

Fig. VI. Second right leg which regenerated from the central region of the second terminal segment or propodite. The propodite was cut at the level (*1db*). The renewed part (*reg*) was regenerated in two months and eleven days. *2*, *3*, and *4* correspond to the same joints as in Fig. III. (All drawings made from life, $\frac{3}{4}$ nat. size.)


I
4
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1
3
II
2
1
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3
III
pr
de
cp
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1db
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2
1
5
bc
cx
IV
reg. is.
V
pc
pb
pa
VI
4
reg.
1db
3
2

OBSERVATIONS ON THE SOFT-SHELL CLAM.

(FIFTH PAPER)

By A. D. MEAD and E. W. BARNES.

Thirty-fourth Annual Report of the Commissioners of Inland Fisheries
of Rhode Island, made to the General Assembly at its
January Session, 1904, p. 29.

OBSERVATIONS ON THE SOFT-SHELL CLAM.

(*FIFTH PAPER.*)

A. D. MEAD AND E. W. BARNES.

Since the year 1898 investigations have been conducted by the commission in regard to the life history of the soft-shell clam (*Mya arenaria*) and the various economic problems in connection with it.

Although there are questions which still remain unanswered, and new ones are constantly arising, many of the problems which were presented at first have been satisfactorily solved. It seems appropriate at this time, therefore, to bring together the results of the several years' work into a connected account.

The present importance and future possibilities of the clam industry in Rhode Island are not generally appreciated. Narragansett Bay has 250 miles of protected shore line, including peninsulas, estuaries, and islands, and is situated in the central portion of the clam's geographical range. At each ebb of the tide over ten thousand acres of available clam ground are uncovered. Rhode Island is famous for its clambakes, and nowhere in the world are clams more tender and palatable. More than a thousand men depend upon this product of the shore for their maintenance. The shores do not now yield the abundance of clams which they did in former years, but we believe that the production can be increased, not only to its former amount, but for beyond it.

The subject will be discussed according to the following outline:

Part I. Life History and Habits.

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The Free-Swimming Period	32
The Attachment Period	34
The Commencement of Burrowing	35
Growth and Age at Sexual Maturity	36

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Physical Causes of the Depletion of Our Clam Flats	37
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Methods of Increasing the Yield of Clams	41
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PART I. LIFE HISTORY AND HABITS.

THE SPAWNING SEASON.

The eggs of the clam, which are extremely minute, are extruded from the siphon, or snout, of the female clam into the water, where they are fertilized by sperm which the male clam extrudes in a similar manner.

The spawning season proper extends throughout the latter part of May and the whole of June, and sometimes into the first of July. At this season the great majority of the eggs are laid.

Mature clams which were examined on the 8th and 12th of May, 1899, were found to have their sexual organs full of nearly ripe sexual products. On the 18th, and again on the 20th, artificial fertilization was attempted, and, although only from ten to twenty per cent. of the eggs were found to develop normally, at no time of year has artificial fertilization of the clam been more successful. These results were confirmed in the following year. This, and the fact that the swimming young were found at this season, shows that the clams were ready to breed during the last of May. The spawning season, however, apparently reaches its height about the middle of June, and clams examined after this time are found, in most cases, to have extruded their eggs; by the first of July the set on the shore is excellent.

There is no doubt, however, that the clam also spawns to some extent throughout the summer and probably until late autumn. This has been proved by the discovery of the very small clams along the shores and in the spat collectors at different times of the year. The clam is not unique in this respect, for many other species, which have a regular breeding season, are known to spawn to a greater or less extent at all times of the year.

In the early spring of 1901, 1902, and 1903 there were discovered sets of clams less than one-quarter of an inch long, which were distinguished from those of the summer sets by having the umbone (that portion of the shell nearest the hinge) raised in relief from the rest of the shell, and showing by its corrosion that the clams had lain for months (i. e. during the winter) with almost no growth. The small size of the clams during this dormant period, as indicated by the corroded part of the shell, was ample proof that they could not possibly have set during the previous summer; for the clams of the previous summer's set averaged 25 mm. (1 inch) in length at the commencement of winter. Throughout the summer the clams of the fall set were readily distinguished from those which set in the spring. Along the shore, this fall's set would scarcely be noticeable, but in the commission's spat collector at Rumstick there were, in the middle of January, 1903, more than one hundred to the square foot. At Kickemuit river, in a spot very favorable for clams, there were at this time hardly more than two or three to the square foot. According to the observations of the last five years the clams which set at any time other than the early summer seem to be of little economic importance.

THE FREE SWIMMING PERIOD.

The eggs of the clam, after being fertilized in the water, develop into free-swimming larvæ which are carried by the tides and scattered in all directions. In this stage they are barely visible to the naked eye. When examined under the microscope they are seen to resemble the adult clams in the possession of a pair of hinged shells, but differ widely in shape and structure. This is very unfortunate for the investigator, because the oyster, quahaug, scallop, and other bivalves breed about the same period, and these also have free swimming larvæ, which in many cases closely resemble those of the clam. A close study of these forms, however, enables one to distinguish by one means or another the oyster and the scallop from the clam, and doubtless sufficient investigation would enable one to identify all the forms. For positive identification certain of these free swimming larvæ, which were supposed to be clams, were kept under observation until they lost their swimming organs and took on the form which could be recognized readily.

In collecting the clams of this stage, a silk bolting-cloth tow-net, which has an extremely fine mesh, is used, and from the first of June until the middle of July these minute clams can be obtained every day by dragging the net along the surface of the water. In order to separate them from the many other small organisms, the dish of sea water into which the contents of the net have been emptied is gently tapped. The clams immediately close their shells and sink to the bottom. The top water is then drawn off, some clear sea water is added, and, whenever the clams are desired, they can be precipitated to the bottom by tapping the dish, and then transferred by means of a pipette to a watch glass under the microscope. As one watches these specimens under the microscope, very frequently he will see one of them open its shell, thrust out and expand a circular swimming organ (*velum*), and go whirling about through the water; then suddenly draw in the velum, close the shell, and fall to the bottom.

Some specimens larger than the average have the foot and siphon well developed, and, when watched under the microscope, are seen to crawl about rapidly, by means of the foot, instead of swimming. The swimming organ gradually disappears while the foot grows, and, at a certain stage, they alternately swim and crawl. The foot soon becomes proportionately very large, and it is not impossible that the clams may sometimes use it in swimming, though this has not been observed.*

The clams often swim about very freely for a week or two after they are captured. Then they lose their swimming organs, the siphon and foot are developed, the shell changes in shape, and the larvæ assume the unmistakable characteristics of the species. Up to this time the transparent shell allows the internal organs to be plainly visible.

The growth at this period is very remarkable. Exactly how fast they grow under the natural conditions is not known, but those which were kept in a dish of water, where the supply of food was meagre as compared with that in the tide water, and where other conditions were necessarily artificial, grew to ten or twelve times their original length in less than two weeks.

The principal changes which may easily be observed in the clam are the elongation of the shell (the outline at first being very much rounded), the increase in the number of gill plates, and the relative lengthening of the foot.

THE ATTACHMENT PERIOD.

The free-swimming period usually ends late in June or early in July. If the eel-grass and sea-weed are carefully examined at this time, large numbers of these little clams may be found hanging to the stalks by the slender gelatinous threads. They attach themselves also to driftwood, spiles, stones, etc. This thread (the byssus)

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*The razor clam (*Ensatella Americana*) has this ability even after it has attained the length of three or four inches, although its foot is aided by forcing backward of water through the mantle opening. On one occasion a large number of specimens about one-quarter of an inch long were caught at the surface.

is elastic and yet very tough, like that of the mussel, and arises from the byssus gland which is situated on the ventral side of the foot near the extreme posterior end. It is an entirely homogeneous structure, and is usually single throughout its entire length, but sometimes near the end it divides into two or more branches. The clam has the ability to free itself from attachment and to form a new byssus at will.

The clam at this period may be recognized readily by its shape, yet it differs in the following manner from the adult. The outline is more rounded, and the umbones are more prominent and farther apart. The foot is very long and capable of quick movements, and extends over the entire surface ventral to the visceral mass, while in the adult clam it is hatchet-like and protrudes only from the extreme anterior end of the visceral mass. The siphon (snout), while quite like that of the adult in shape, is relatively much smaller, and consequently the shell does not gape open so much posteriorly. The siphon, like the foot, is more easily and rapidly protracted and retracted than in the adult.

The growth of the clam is still very rapid, and it is during this period that it assumes the shape of the adult. One remarkable fact in this change is the double shiftng of the position of the umbones, or hinge prominences. It will be recalled that, in the adult clam, the position of the umbones is nearly central. This is also the case with the very small clam, but, through an increased growth posteriorly, the umbones become shifted more and more anteriorly till the clam is about 6 mm. long; then, by increased anterior growth, the umbones again gradually assume a central position.

The duration of this attachment period seems to depend, to a certain degree, on the environment. Certainly it is not dependent entirely on size, for frequently clams measuring half an inch in length are found attached. The great majority, however, reach the ground and burrow long before attaining this size. The byssus is retained some time after the clam has left its place of attachment and has buried itself in the soil; by means of it the clam attaches itself to grains of sand within its burrow and thus anchors itself.

THE COMMENCEMENT OF BURROWING.

Clams begin to burrow as soon as they find a favorable location. This may be soon after they cease swimming, or, if they have a chance to settle upon sea-weed or other objects not near the ground, it may be weeks before the burrowing actually begins. Even after they have burrowed, they seem for a time to be restless, and, as first observed by Kellogg, the small clams, even those sometimes an inch and a half in length, will leave their burrows without apparent reason and wander about. Kellogg saw one such specimen try six different places in three days. Usually before they reach this size, however, they are permanently located and evidently satisfied to pass their allotted years in one place. The period of commencement of burrowing is a critical one for many reasons. The young clams cannot live far below the surface, and though they may anchor themselves within their burrows, they are still liable to be washed out by storms, crowded out by their neighbors if the set is very thick, or dug out by crabs, eels, duck, and other enemies. It often happens, too, that localities which are particularly favorable for setting are entirely unfit for subsequent growth. Specific instances have not infrequently come to our notice in which abundant sets have practically been exterminated by decaying sea-weed or shifting sand.

GROWTH AND AGE AT SEXUAL MATURITY.

Just how long the clam lives has not yet been determined, owing to the difficulty in finding reliable marks of age. The growth during the first few years has been carefully traced in our experiments, and it is evident that the growth becomes slower with advancing age. Considering the very large size of clams frequently found in flats which are exposed only at extremely low winter tides, together with the relatively slow growth of old clams as shown in other observations, it would seem that clams, like some other marine animals, may live until some accident deprives them of life. Some of the clams raised from the seed at the Wickford Experiment Station are now in their fourth year, and do not show any signs of degeneration.*

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* For definite data concerning the rate of growth, refer to page 54.

PART II. QUESTIONS OF ECONOMIC IMPORTANCE.

The purpose of the inquiry into the life history and habits of the clam has been to ascertain how to control the conditions upon which the yield of clams depends. The scientific results have indicated the solution of the practical problems at every step. From the economic point of view there are two general problems, viz., the causes of the present depleted condition of our clam flats, and the means of restoring or increasing the natural yield.

PHYSICAL CAUSES OF THE DEPLETION OF THE CLAM BEDS.

In view of the many adverse physical conditions and natural enemies with which the clam has to contend from the time the egg is laid until maturity, the chances of an individual's reaching the adult condition would seem to be almost infinitesimal. The minute, unprotected eggs, extruded wherever the female may happen to have burrowed, lie upon the ground or are swept into the current, and there await the arrival of the spermatozoa which are extruded into the water by the male. It is, of course, impossible to say how great are the chances of non-fertilization.

When the eggs have developed into swimming larvæ they are still at the mercy of the tides; they may be carried out to sea or lodged in muddy, rocky, or otherwise impossible places. Those which have successfully set and even burrowed may still be destroyed by shifting sand or silt, or washed out of their burrows and exposed to their many enemies. At times clams, an inch or more in length, are found dead in immense numbers, apparently from the effects of the excessive heat, where the shore is underlaid with hard clay and covered with a thin layer of sand in which the clams have burrowed. At Pawtuxet, during the past year, great quantities of clams were found dead, and several visits to the flats failed to reveal any other cause than the one just suggested.

Not infrequently clams in a particular region appear to die of an epidemic disease that cannot be referred to the heat. Some small experimental beds at Wickford were completely destroyed in this way, the clams dying, all nearly at the same time, and rotting in their burrows. The subject of clam diseases has yet to be investigated.

On one occasion, at least, the presence of the red-water in great quantity seems to have been the direct cause of the destruction of vast quantities of clams. About the first of October, thousands of bushels of clams about an inch in length were washed ashore on the beach about an eighth of a mile north of the Rhode Island Yacht Club, at Pawtuxet, and when they were examined a considerable portion of them were still alive. There had been, of course, no excessive heat, and no storm of sufficient violence, in our opinion, to wash the clams out of their burrows; but there had been, recently, a phenomenal quantity of peridinium in that vicinity, and we are strongly inclined to believe that the latter were the cause of this wholesale destruction. It is, indeed, characteristic of most animals, shrimp, crabs, flatfish, etc., in the presence of this plague, to endeavor to come to the surface, and when they are first washed ashore they are, as a rule, still alive.

NATURAL ENEMIES OF THE CLAM.

Clams are beset, from the very beginning, by numerous enemies. The eggs and free-swimming fry furnish food for many varieties of fish, mollusks, crustacea, etc. As soon as they cease swimming and commence to burrow, the young star-fish, mummychogs, crabs, and "oyster drills" feed upon them.

It is the universal verdict of fishermen that the paddler crab (*Callinectes hastatus*) is a most serious menace to the young clams; and, though experiments have been tried with this species, which have given only a negative result, the rock crabs (*Cancer irroratus*) were found to dig up and eat clams of considerable size. In one of our experiments four of these crabs dug up and made way with twenty-one fair sized clams in the course of two weeks.

The Sea Snail (*Neverita duplicata*). Often along our shore clam shells are found with holes neatly bored through them, usually near the hinge, which resemble those frequently found in quahaug shells. The latter are easily traced to the "cockle," or sea snail (*Neverita*). It would be natural, judging from the similarity of the perforations in the shell of the clam and the quahaug, to assign them both to the same cause, except for the fact that clams lie buried in from six to twelve inches of soil, while the *Neverita* and quahaug are usually found near the surface. The *Neverita*, however, actually burrows after the clams, and a specimen was found at Wickford six inches deep in the firm soil in the act of boring a clam. The hole had been drilled about half way through the shell. Following this discovery some experiments were made to test the matter further. A *Neverita*, placed in an inverted orange box over one of our clam beds, bored and devoured seven good sized clams in two weeks, and, at the end of the experiment, was found five inches deep in the soil.

The *Neverita* are not, perhaps, numerous enough in our bay to be a very serious injury to the clam product, but in Plymouth, Mass., they are excedingly abundant and are collected and sold for bait. In the year 1901, 8,000 buckets of these animals, which go there by the name of cachels, were collected and sold at Plymouth. They bring from sixty to seventy-five cents a bucket, and the catch, therefore, amounted to nearly 6,000 dollars. During the past year about 4,000 bushels were "picked."

There are other gasteropods which drill holes in the shells of clams, especially in the young ones; for example, the oyster drill (*Urosalpinx*). Those clams which have come to the surface of the ground or have been forced out of their burrows are especially attacked by these animals, and often the shores are strewn with the bored shells. The holes can easily be distinguished from those bored by the *Neverita* on account of their small diameter. Fig. 7 is from a life size photograph showing a series of bored clams, the work both of the oyster drill and of the *Neverita*.

Man as an Enemy of the Clam.—After all has been said concerning the physical conditions and destructive enemies of the clams, the proposition remains that the clams continued to hold their own in abundance along our shores until a comparatively recent date. Though the chances of death of individuals are so great, nature has provided an ample protection for the species in the tremendous productivity of the clam. Were the chances against reaching maturity millions to one (and they probably are), the eggs are laid in sufficient abundance to offset them.

But in recent years the clams have not held their own, and the decrease is, without question, the direct result of the promiscuous, unlimited, and unreasonable digging by man. There is no evidence that the physical conditions or natural enemies have recently become more destructive, nor can more than a small part of the responsibility be laid at the door of the manufacturers who allow detrimental waste products to pour into the bay. The decrease is quite as well marked in regions where the water is good and where clams, if left to themselves, thrive well. The decrease has been assigned also to such causes as the wash of steamers which ply the waters of the bay more frequently and more rapidly than in former years, but the same answer applies to this suggestion, viz.: the clams have decreased on shores which are not washed by the swell of steamers. But where is the square yard of shore, known to contain clams, which has not been dug over and over again, and all the clams found, which were more than an inch and a half long, taken out? Not only are all the clams of edible size taken, if discovered, but the smaller ones are, many of them, buried deep or washed out and left exposed to natural enemies.

The practice of continually digging the shores is defended by many clammers, on the ground that it is good for the clams or even necessary to their growth, but our observations and experiments argue against this proposition, except in rare cases where the set is so exceedingly thick that the individuals actually crowd one another. Time and again in experiments on a small scale, and in some larger experiments, like those at Kickemuit river and Wickford, clams have been planted and left undisturbed and have grown remarkably well. See page 67.

It is a belief almost universally held that the soil loosened by much digging is rendered favorable for the setting of clams, but even this proposition our experiments contradict. The remarkably thick natural sets of small clams found in different years were, in every instance, upon shores which contained so few old clams that they were not dug over. For example, the point of Cornelius Island, at Wickford, yielded 12,500 clams to the square yard; the point at Old Buttonwoods, in 1900, yielded nearly as many; the east shore of Green's Island in 1891, yielded about 8,000 clams to the *square foot;* the same point on Cornelius Island, again in 1900 yielded 7,715 clams per square yard. These areas of shore were, at the time when the clams set, lying undisturbed. On the other hand, we have not found sets nearly so dense in any area which had been dug over, although of course good sets have been deposited in such places. Again, in the commission's bed, at the Kickemuit river, which rested undisturbed for two years, not only was there an excellent product from the clams which were sown, but there was also a heavy set of new clams.

We are convinced that the clam in Narragansett Bay can, at the present time, as in the past, hold its own against natural enemies and adverse physical conditions, and can regain and maintain its former abundance, and, moreover, that excessive digging is the immediate cause of the decrease of the product.

METHODS OF INCREASING THE YIELD OF CLAMS.

In developing methods of clam culture we have constantly kept in mind two possibilities: that of reinstating the clams on the shores for public digging, and that of cultivating them on grounds leased to private parties. Success in either case must obviously depend on the degree to which unfavorable conditions of life are reduced and favorable ones increased.

Increase in the Production of Spawn.—That the general increase in the amount of spawn and the chances of fertilization would follow a general increase in the abundance of adult clams is self evident. Experiments in artificial fertilization have been tried many times and a sufficient number of eggs were fertilized for working out the early stages of development, but as a practical method it does not yet give promise of success.

The Increase in the Production of the Set.—A good deal, we believe, can be accomplished at this point. It is a significant fact that the water is, at certain times of the year, swarming with clam larvæ ready to set. A phenomenon witnessed almost every year of remarkably thick sets in certain limited areas, and the fact that equally thick sets have been caught by artificial contrivances in places where the natural set was sparse, shows that favorable conditions at the place of setting is the most important factor, and probably one which may be, to a certain extent, controlled.

Of the spat collectors referred to, two forms have worked successfully. The first, described more fully in the report for 1900, consisted of a small, square, bottomless box, 12 by 14 inches in diameter, set firmly into the ground near the low-water mark, and covered with a fine galvanized wire netting, "pearl cloth." The swimming fry apparently hit upon the netting and fall to the ground, and, if they rise again as far as the netting, they are apt to be again precipitated. In this box we caught about 15,000 clams. Another successful form is that installed at Mr. Alexander's estate, on Rumstick Point (described and figured in the last report). It consists of a sand-filled box with a wooden cover provided with a window of pearl cloth and bored with numerous small holes. A large number of clams have been caught in this apparatus.

The Protection of Spawn and Swimming Fry.—To protect the spawn and swimming fry from adverse physical conditions and natural enemies is, as far as we know, impossible. These microscopic creatures are carried hither and thither, according to the condition of tides and winds, light and darkness, heat and cold—for they doubtless rise and sink in response to changes in light and in temperature like many other minute swimming forms. They are no more likely to return to the flats where they were produced than to settle in distant places, and, therefore, a man who should keep a bed of breeding clams would not benefit himself any more than his neighbors. We cannot control these conditions, and no more can we control the activities of the myriads of minute animals which feed on the swimming clams, such, for example, as the worm larvæ, copepods, crab larvæ, and hundreds of species, not commonly known, which abound in the waters of the Bay.

Protection of Spat.—When the clam is finally set and the spat begins to burrow, something can be accomplished toward their protection; chiefly, however, by protecting them from the clam hoe, and in transplanting them when they have set too thickly.

TRANSPLANTING.

A large number of experiments have been conducted, during the last few years, which have demonstrated that it is perfectly feasible to transplant clams and have given definite information in regard to the following points: the best size for planting, the methods of collecting and caring for the seed between the time of collecting and planting, the value of different soils, the methods of planting, the rate of burrowing, the rate of growth, and the like.

Best Size for Transplanting.—Clams of any size, from one-eighth of an inch to five inches in length, can be, and in our experiments have been, successfully transplanted. The smaller sizes, one-half inch more or less in length, are best for several reasons: They can usually be obtained more easily than the larger ones, and they burrow more rapidly and more surely; they can, indeed, be sown upon the surface of the ground, like oats, so great is their capacity for burrowing. This burrowing power decreases with the size of the clam. The growth of the young clams is much more rapid than that of the old ones. Finally, if they are left undisturbed, they grow to a large size, with a handsome shell, even and smooth.

Methods of Collecting Seed.—Where the small clams can be found in great abundance there is no difficulty in procuring seed by the use of large sieves. In three of the four seasons in which we have sought such places in Narragansett Bay, they have been found. The year 1902 was an exception, and no very large sets were found, although the shores were carefully examined. The best time for collecting such seed is from the middle of July to the first of September. If the spat collectors, so successful in the small experiments, succeed on a large scale, an unlimited supply of seed can be secured in this way.

It is, of course, by no means unprofitable to plant clams of larger size, if the smaller ones cannot be obtained.

Methods of Caring for Seed Clams.—From the time the clams are dug until they are planted again they are best kept out of water, in thin layers to allow ventilation, and they should not, of course, be exposed to the sun. If they are small, they should not be allowed to become too dry, for it is not only actually injurious, but the much dried specimens will float away on the surface of the water. They can be kept well in moist sea weed.

At first thought it would seem to be better to keep them in fresh seawater, if possible in cars, but experiments show that the clams kept in this way burrow far less rapidly. Above all it is important to plant the clams as soon as possible after they have been dug; if they are kept, even for a day or two, they begin to lose, very noticeably, their power of burrowing.

Soil.—Clams will live and thrive in nearly every kind of soil; sticky mud and shifting sand, of course, must be avoided. In stony ground the shells are apt to become badly contorted, and even in gravelly soil the shells usually grow heavy and are marred by numerous so-called growth rings. These rings are produced by the wearing off of the thin edge of the shell; and in gravel, especially, if the clams are from time to time disturbed, this often occurs. Similar rings have been repeatedly produced artificially by running a file over the edge of the shell and then planting the clam; and the same principle applies to the notched clams described on page 54.

Method of Planting.—At this point there are many things which must be taken into consideration; the size and condition of the clams, the kind of soil, the time of day, time of tide, etc. The main point in planting is to get the largest possible proportion of the clams well installed in their burrows, with heads down and in firm soil. The method of sowing clams on the surface has been tried with a good degree of success, especially in the case of small clams measuring up to an inch, more or less, in length. Experiments tabulated on pages 47 and 64 represent very well the usual result of the sowing method. It will be seen by consulting this table that much depends upon the condition of the soil and upon the condition of the clams. The method has two distinct advantages; first, it is very rapid, and, second, when the clams have burrowed, they are in their proper position.

The advisability of loosening the soil before sowing is in some cases, perhaps, doubtful. Certainly the clams burrow more rapidly; but on the other hand some soils are made rather soft and watery when they are dug over and require considerable time to become firm again. In such cases the clams are more easily reached by predaceous crabs and eels, and washed out by the waves.

Temperature.—In cold weather the clams, like many other cold blooded animals, are torpid, and they burrow more slowly than usual, and sometimes not at all. Of a bushel of small clams, three-quarters of an inch in length, which were sowed on a cold day about the first of November, 1903, only very few burrowed during the first hour, most of them remaining on top of the ground until nearly noon the next day, when, as the weather became warmer, they began to burrow.

Time of Day and Time of Tide.—Clams sown with the rising tide have the advantage of an immediate chance to burrow, whereas those sown on the bare ground rarely succeed in burrowing until the tide has risen and covered them. It is not advisable to plant clams in the late afternoon, because, with the approach of darkness, the eels, crabs, and other enemies come in shore. We have often sowed them in the water over the beds with good results.

Tabulated Results of Experiments Upon the Rate of Burrowing Under Different Conditions.—The tables which are here republished are arranged from careful experiments made in the summer of 1900, and subsequent practice in planting has given them general substantiation.

The clams selected for these experiments were separated into four classes which are given successively in the tables, viz.:

I. Large, 50-70mm. (2–2¾ inches) in length;
II. Medium, 35-50mm.;
III. Small, 25-35mm.;
IV. Small set of 1900, 20-30mm.

Set of 1899 or previous to that date.

To insure the protection of the clams while exposed, and to prevent them from washing away, boxes with 8-inch sides and ½-inch wire mesh bottoms were inverted over the beds. All the clams were sowed on the surface just as the tide was beginning to rise.

The attempt was made to determine the value of the following conditions for each class of clams:

a. Sowing clams immediately after digging.
b. " " kept 24 hours out of water (dry).
c. " " " 24 " in sea-water.
d. " " in soil softened by previous digging.
e. " " " not dug up.
f. " " in gravel.
g. " " in sand.

For convenience in referring to the tables, these letters are placed in the first column.

An examination of the experiments here tabulated shows some interesting results which may be stated briefly as follows:

Of the three classes of clams which were more than one year old the smaller specimens were most successful in burrowing, the middle ones next, and the largest clams least successful. The fourth class composed of specimens a few months old were slightly less successful than the third.

Of the I (50-70mm. 2–2¾ inches) class, under all conditions	70	per cent.	burrowed.
II (35–50mm. 1⅜–2 inches)	85	per cent.	"
III (25–35mm. 1–1⅜ inches)	90.5	per cent.	"
IV (20–30mm. ¾–1⅜ inches)	87.7	per cent.	"

The effects of the varying conditions may be stated as follows:

(*a*) Planted immediately after digging (1 hour or less):

Class I	72	per cent.	burrowed.
" II	96	per cent.	"
" III	100	per cent.	"
" IV	97	per cent.	"

Average per cent. = 91.2

(*b*) Dry 24 hours.

Class I	68	per cent.	burrowed.
" II	78.6	per cent.	"
" III	97.3	per cent.	"
" IV	96	per cent.	"

Average per cent. =84.9

(*c*) Kept in water 24 hours between digging and sowing.

Class I	70.6	per cent.	burrowed.
" II	84.0	per cent.	"
" III	73.3	per cent.	"
" IV	73.3	per cent.	"

Average per cent. = 76.3

(*d*) Soil dug up before sowing.

Class I	82.6	per cent.	burrowed.
" II	94.6	per cent.	"
" III	97.3	per cent.	"
" IV	92	per cent.	"

Average per cent. = 91.6

(*e*) Soil not dug up before sowing.

Class I	62.4	per cent.	burrowed.
" II	79.2	per cent.	"
" III	86.4	per cent.	"
" IV	85.2	per cent.	"

Average per cent. = 78.3

(*f*) Sand.

Class I	70.6 per cent.	burrowed.
" II	88 per cent.	"
" III	92 per cent.	"
" IV	88.6 per cent.	"

Average per cent. = 84.8

(*g*) Gravel.

Class I	68 per cent.	burrowed.
" II	76 per cent.	"
" III	86 per cent.	"
" IV	84.5 per cent.	"

Average per cent. = 78.7

Summary: The average per cent. of all clams	83.4 burrowed.
The average per cent. of those planted immediately after digging	91.2
" " of those dry 24 hours after digging	84.9
" " of those in water 24 hours after digging	76.3
" " of those in soil dug up before sowing	91.6
" " of those in soil not dug up before sowing	78.3

The details of these experiments are tabulated in the succeeding pages:

CLASS I.	Date.	Hour.	Number sowed.	Year of set.	Length in mm.	Time out of soil	How kept.	Soil.	Soil dug up?	Date of examination.	Hour of examination.	Time between sowing and examination.	Number exposed.	Per cent. burrowed.
a. d.	Sept. 13	4-5 P.M.	25	1898?	50-70	abt. 1 hour	dry	sand	yes	Sept. 14	8-9 A.M	16 hours	11	(56)
										Sept. 14	5 P.M.	24 hours	8	68
a. e.	Sept. 13	4-5 P.M.	25	1899?	50-70	1 hour	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	9	(64)
										Sept. 14	5 P.M.	24 hours	6	76
b. d.	Sept. 13	4-5 P.M.	25	1899?	50-70	24 hours	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	8	(68)
										Sept. 14	5 P.M.	24 hours	2	92
b. e.	Sept. 13	4-5 P.M.	25	1899?	50-70	24 hours	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	18	(28)
										Sept. 14	5 P.M.	24 hours	13	48
c. d.	Sept. 13	4-5 P.M.	25	1899?	50-70	24 hours	water	sand	yes	Sept. 14	8-9 A.M.	16 hours	8	(68)
										Sept. 14	5 P.M.	24 hours	3	88
c. e.	Sept. 13	4-5 P.M.	25	1899?	50-70	24 hours	water	sand	no	Sept. 14	8-9 A.M.	16 hours	16	(36)
										Sept. 14	5 P.M.	24 hours	12	52
b. e.	Sept. 15	6-6.30 P.M.	25	1899?	50-70	24 hours	dry	gravel	no	Sept. 16	9.30 A.M.	15 hours	13	(48)
										Sept. 17	8 A.M.	40 hours	9	64
c. e.	Sept. 15	6-6.30 P.M.	25	1899?	50-70	24 hours	water	gravel	no	Sept. 16	9.30 A.M.	15 hours	17	(32)
										Sept. 17	8 A.M.	40 hours	7	72
											Average percentage		=	70

CLASS II.	Date.	Hour.	Number sowed.	Year of set.	Length in mm.	Time out of soil.	How kept.	Soil.	Soil dug up?	Date of examination.	Hour of examination.	Time between sowing and examination.	Number exposed.	Per cent. burrowed.
a. d.	Sept. 13	4-5 P.M.	25	1899?	35-50	1 hour ±	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	4	(84)
										Sept. 14	5 P.M.	24 hours	2	92
a. e.	Sept. 13	4-5 P.M.	25	1899?	35-50	1 hour ±	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	4	(84)
										Sept. 14	5 P.M.	24 hours	0	100
b. d.	Sept. 13	4-5 P.M.	25	1899?	35-50	24 hours	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	3	(88)
										Sept. 14	5 P.M.	24 hours	1	96
b. e.	Sept. 13	4-5 P.M.	25	1899?	35-50	24 hours	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	13	(48)
										Sept. 14	5 P.M.	24 hours	10	60
c. d.	Sept. 13	4-5 P.M.	25	1899?	35-50	24 hours	water	sand	yes	Sept. 14	8-9 A.M.	16 hours	4	(84)
										Sept. 14	5 P.M.	24 hours	1	96
c. e.	Sept. 13	4-5 P.M.	25	1899?	35-50	24 hours	water	sand	no	Sept. 14	8-9 A.M.	16 hours	7	(72)
										Sept. 14	5 P.M.	24 hours	4	84
b. e.	Sept. 15	6-6.30 P.M.	25	1899?	35-50	24 hours	dry	gravel	no	Sept. 16	9.30 A.M.	15 hours	10	(60)
										Sept. 17	8 A.M.	40 hours	5	80
c. e.	Sept. 15	6-6.30 P.M.	25	1899?	35-50	24 hours	water	gravel	no	Sept. 16	9.30 A.M.	15 hours	12	(52)
										Sept. 17	8 A.M.	40 hours	7	72
											Average percentage		=	86¼

Class III.	Date.	Hour.	Number sowed.	Year of set.	Length in mm.	Time out of soil.	How kept.	Soil.	Soil dug up?	Date of examination.	Hour of examination.	Time between sowing and examination.	Number exposed.	Per cent. burrowed.
a. d.	Sept. 13	4-5 P.M.	25	1899	25-35	1 hour or less	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	1	(96)
										Sept. 14	5 P.M.	24 hours	0	100
a. e.	Sept. 13	4-5 P.M.	25	1899	25-35	1 hour or less	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	1	(96)
										Sept. 14	5 P.M.	24 hours	0	100
b. d.	Sept. 13	4-5 P.M.	25	1899	25-35	24 hours	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	1	(96)
										Sept. 14	5 P.M.	24 hours	0	100
b. e.	Sept. 13	4-5 P.M.	25	1899	25-35	24 hours	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	4	(84)
										Sept. 14	5 P.M.	24 hours	2	92
c. d.	Sept. 13	4-5 P.M.	25	1899	25-35	24 hours	water	sand	yes	Sept 14	8-9 A.M.	16 hours	6	(76)
										Sept. 14	5 P.M.	24 hours	2	92
c. e.	Sept. 13	4-5 P.M.	25	1899	25-35	24 hours	water	sand	no	Sept. 14	8-9 A.M.	16 hours	14	(44)
										Sept. 14	5 P.M.	24 hours	8	68
b. e.	Sept. 15	6-6.30 P.M.	25	1899	25-35	24 hours	dry	gravel	no	Sept 16	9 30 A M.	15 hours	5	(80)
										Sept. 17	8 A.M.	40 hours	0	100
c. e.	Sept. 15	6-6.30 A.M.	25	1899	25-35	24 hours	water	gravel	no	Sept. 16	9.30 A.M.	15 hours	13	(48)
										Sept. 17	8 A.M.	40 hours	7	72
											Average percentage		=	90½

CLASS IV.	Date.	Hour.	Number sowed.	Year of set.	Length in mm.	Time out of soil.	How kept.	Soil.	Soil dug up?	Date of examination.	Hour of examination.	Time between sowing and examination.	Number exposed.	Per cent. burrowed.
a. d.	Sept. 13	4-5 P.M.	50	1900	20-30	1 hour or less	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	4	(92)
										Sept. 14	5 P.M.	24 hours	3	94
a. e.	Sept. 13	4-5 P.M.	50	1900	20-30	1 hour or less	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	2	(96)
										Sept. 14	5 P.M.	24 hours	0	100
b. d.	Sept. 13	4-5 P.M.	50	1900	20-30	24 hours	dry	sand	yes	Sept. 14	8-9 A.M.	16 hours	9	(82)
										Sept. 14	5P.M.	24 hours	3	94
b. e.	Sept. 13	4-5 P.M.	50	1900	20-30	24 hours	dry	sand	no	Sept. 14	8-9 A.M.	16 hours	8	(84)
										Sept. 14	5 P.M.	24 hours	2	96
c. d.	Sept. 13	4-5 P.M.	50	1900	20-30	24 hours	water	sand	yes	Sept. 14	8-9 A.M.	16 hours	9	(82)
										Sept. 14	5 P.M.	24 hours	6	88
c. e.	Sept. 13	4-5 P.M.	50	1900	20-30	24 hours	water	sand	no	Sept. 14	8-9 A.M.	16 hours	21	(56)
										Sept. 14	5 P.M.	24 hours	20	60
b. e.	Sept. 15	6-6.30 P.M.	50	1900	20-30	24 hours	dry	gravel	no	Sept. 16	9.30 A.M.	15 hours	3	(94)
										Sept. 17	8 A.M.	40 hours	1	98
c. e.	Sept. 15	6-6.30 P.M.	50	1900	20-30	24 hours	water	gravel	no	Sept. 16	9.30 A.M.	15 hours	23	(54)
										Sept. 17	8 A.M.	40 hours	14	72
											Average percentage	=		87¾

When clams are planted and covered with soil, instead of being sowed on the surface, they should, if possible, be placed in the natural position, head down; otherwise they find difficulty in adjusting themselves, and often even work their way to the surface and burrow again. They should not be planted too deep, for with a considerable depth of soil over them they are apt to smother before they can adjust themselves and reach the surface with their siphons.

An experiment was made in 1902 with three hundred selected clams, one and one-quarter inches in length, for the purpose of demonstrating the effect of planting in different positions and in various depths of soil; and although the experiment was on a small scale, the results indicated clearly the advantage of planting in normal position and not too deep.

Depth as a Factor in Planting.—Results Four Days After.

Depth of Planting.	Method of Planting.	Number Planted.	Number Alive.	Number Dead.	Number Missing.	Range in Depth.	Average Depth.
Just covered over.......	Inverted......	25	22	3	None.	1″-3″	2″
	Buried flat...........	25	24	1	None.	1″-3″	2″
	Normal position......	25	25	0	None.	½-1″-4″	2½″
Six inches...............	Inverted..............	25	17	8	None.	4″-6″-7″	6″
	Buried flat...........	25	19	6	None.	3″-5″-7″	5½″
	Normal position......	25	23	2	None.	1″-3″-5″	4″
Twelve inches....	Inverted..............	50	10	37	3	10″-5″-4″	7″
	Buried flat...........	50	21	29	None.	5″-6″-9″	8″
	Normal position......	50	33	17	None.	1″-4″-8″	6″

The ideal method of planting in case of the larger clams, one and one-half inches long and upwards, is to place the clam head down in a hole made in the firm soil and to cover with an inch or two of loose soil. This, of course, is a rather tedious method for use on a large scale, and yet, if narrow furrows are cut in the ground, a large quantity can be planted in a few hours.

THE RATE OF GROWTH AND AGE AT MATURITY.

In a consideration of clam culture, of the propagation of clams on public shores, or of restrictive legislation, it is of importance to know the rate of growth of the clam, and the age at which it may reasonably be expected to reach a marketable size. This was one of the first questions which the commission undertook to solve; for in regard to it, even among professional clam diggers, there existed a wide difference of opinion. The problem turned out to be less simple than it might seem at first, mainly from the fact, which was soon learned, that the rate of growth varies extremely according to different conditions.

The main spawning season is about June, and the clams ordinarily reach sexual maturity and begin to spawn in one year. They become a marketable size, that is about three inches in length, in from eighteen months to two years, if they are given fairly favorable conditions. In Narragansett Bay, however, they are not intentionally allowed to reach this size, but are dug and sent to market or to the clambakes as soon as they are a year old, and often before. At some of the popular clambakes great quantities of clams are daily served which are not as large as some specimens which we know to be less than four months old.

Methods of Determining the Rate of Growth.—To ascertain the rate of growth, various methods have been employed. Individual specimens have been isolated in flower pots and land tiles which were set into the ground; enclosures of various sorts have been planted with clams, all of approximately the same size; the set of a particular season has been kept under constant observation without transplanting; and large areas of ground, half an acre or more in extent, have been planted in various places.

During the last year a new and excellent method of indelibly marking the shell was devised, which serves as a record of the growth of individual clams wherever they are planted. The so-called rings of growth, usually present on the shell, are not by any means a reliable way of determining the age of the specimen. These rings are due to the rubbing and breaking of the fine edge of the shell from any cause whatever; they are liable to be repeated at any time within a few months, and, on the other hand, they may not appear at all, even in clams which are much more than a year old.*

Distinct rings are always produced when a clam is taken and allowed to burrow again in coarse sand or gravel. We have often produced the ring in this way and also by wearing off the edge of the shell by running a file over it. It was this observation, indeed, which suggested the following simple method of indelibly marking the clams. A small notch is filed into the edge of the shell and the clam returned to the soil. As it grows the notch remains perfectly distinct and always at the original distance from the hinge. When the clam is again examined the notch identifies it as the one which was planted, and furthermore indicates the amount of growth. A growth ring usually accompanies the notch, and so, after a month, or even years, the complete outline of the clam at the time of notching can be readily identified and traced upon the shell of larger growth. The device allows greater freedom in distributing experimental seed clams, and provides, at the same time, perfect accuracy in the results. Figs. 8, 9, 10, and 11.

Experiment Upon Rate of Growth.—The following brief account of some experiments and observations, made during the past few years, gives numerous examples of the sizes of clams at different ages, and illustrates also the variability in the rate of growth. As far as possible the general statements and the statistical tables will be given separately.

Experiment No. 1. An exceedingly thick set of clams, discovered in July, 1899, on the southernmost point of Cornelius Island,

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* Fig. 12 illustrates this point. It is from a life-size photograph of a clam one year and three months old, three inches in length, which, from July 18, 1901, to September 10 of the following year, lay buried in a land tile which was set perpendicularly in the ground. This specimen was not disturbed during this time, and no growth rings are visible on the shell.

Wickford, was kept under observation throughout the summer. On the 9th of August, after they had become considerably thinned by the crowding out process, 12,500 specimens were gathered from one square yard by means of a rather coarse sieve. A large number of smaller specimens were probably lost through the meshes of the sieve. On various dates the average size was calculated from a considerable number of specimens. When first observed, on July 4th, the average length was 6.1mm. (¼ of an inch); a month later, on August 4th, they had more than doubled this length and measured 13.9mm. (½+ inches); on September 30th, the average length was 23.7mm. (⅞ inches); they were then about three months old.

Experiment No. 2. On July 6th and 9th of the same year, clams to the total amount of a pint and a half (about 2,000 specimens) were taken from the above set and sown on the surface of a box of sand and distributed as evenly as possible; the box was kept suspended about 18 inches below the surface of the water at the houseboat. These clams were continuously submerged during the months of July and September, and during August were exposed at low tide occasionally, when the houseboat was ashore. They grew very much faster than those which remained on the shore. For example, on August 4th they averaged 21.8mm. (13-16 inches) in length against 13.9mm. (½ inch) on the shore; and on September 30th 29mm. (1⅛ fnches) against 23.7mm. (⅞ inches) on the shore. The difference was due to the more favorable location of the transplanted clams; they were entirely unmolested and were continuously under water in the tidal current, which was full of food, and they lay night and day with the siphons expanded at the surface of the ground. The two accompanying curves represent the difference in the rate of growth between these two classes of clams.

Experiment No. 3. Several specimens were taken from this same set on July 19th, 1899, individually measured, and kept separate in flower pots placed under water at the houseboat. These specimens measured from 8 to 12mm. (5-16—7-16 inches). About seven weeks later, on September 10th, they were again measured,

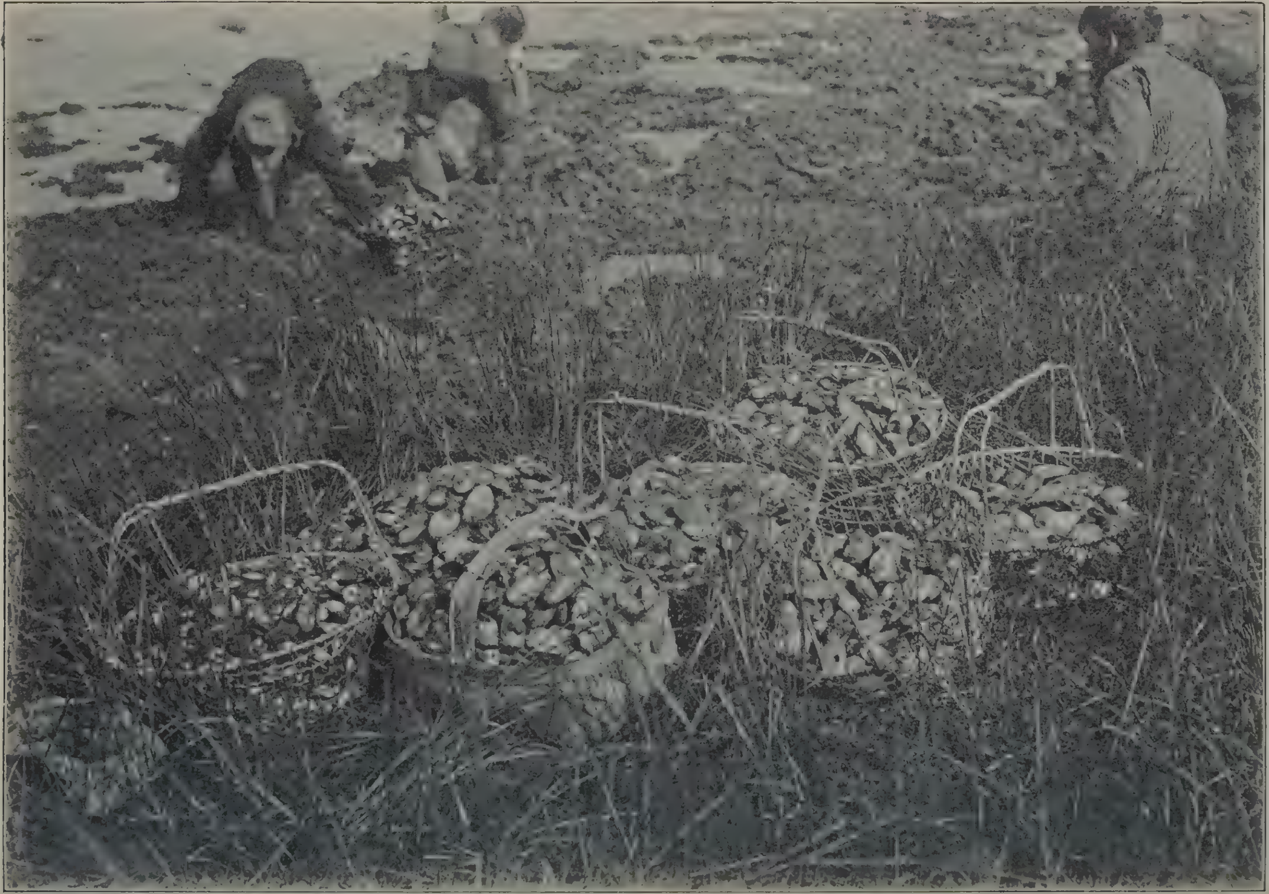


Fig. 1.—A sample digging from the Commission's bed at Kickemuit river

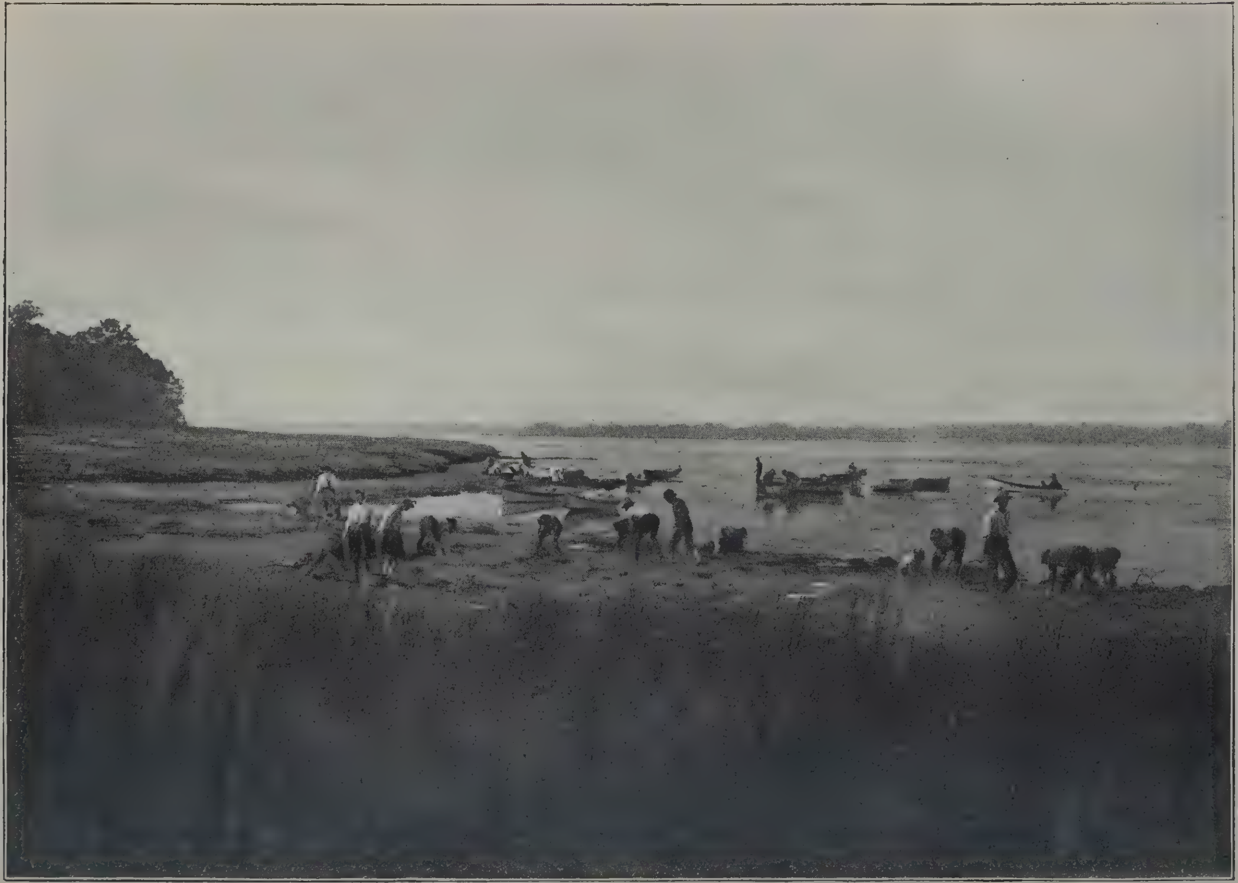


Fig. 2.—Digging clams at the Commission's clam bed, at Kickemuit river.

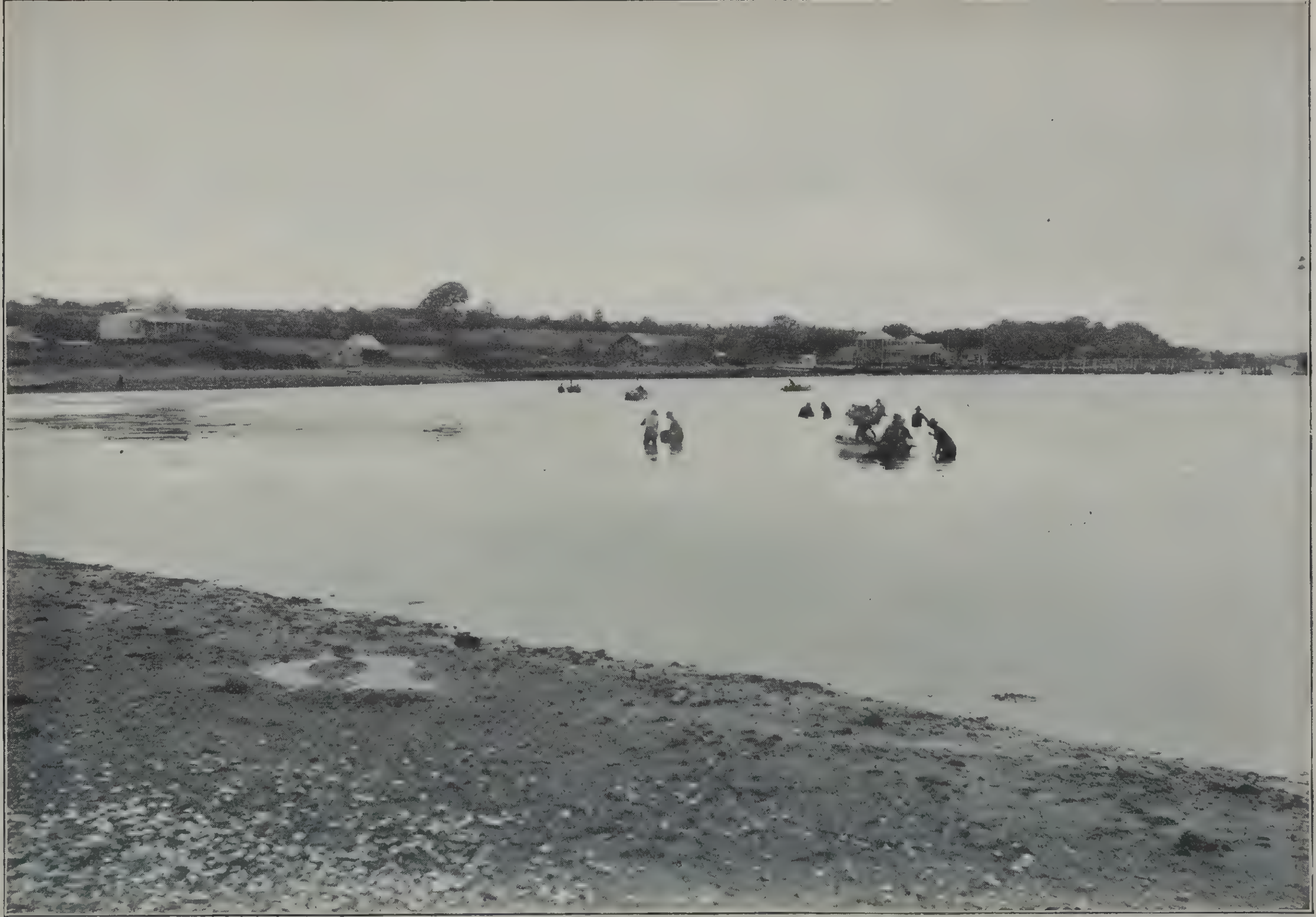


Fig. 8.—Churning clams in the Commission's clam bed at Kickemuit river.

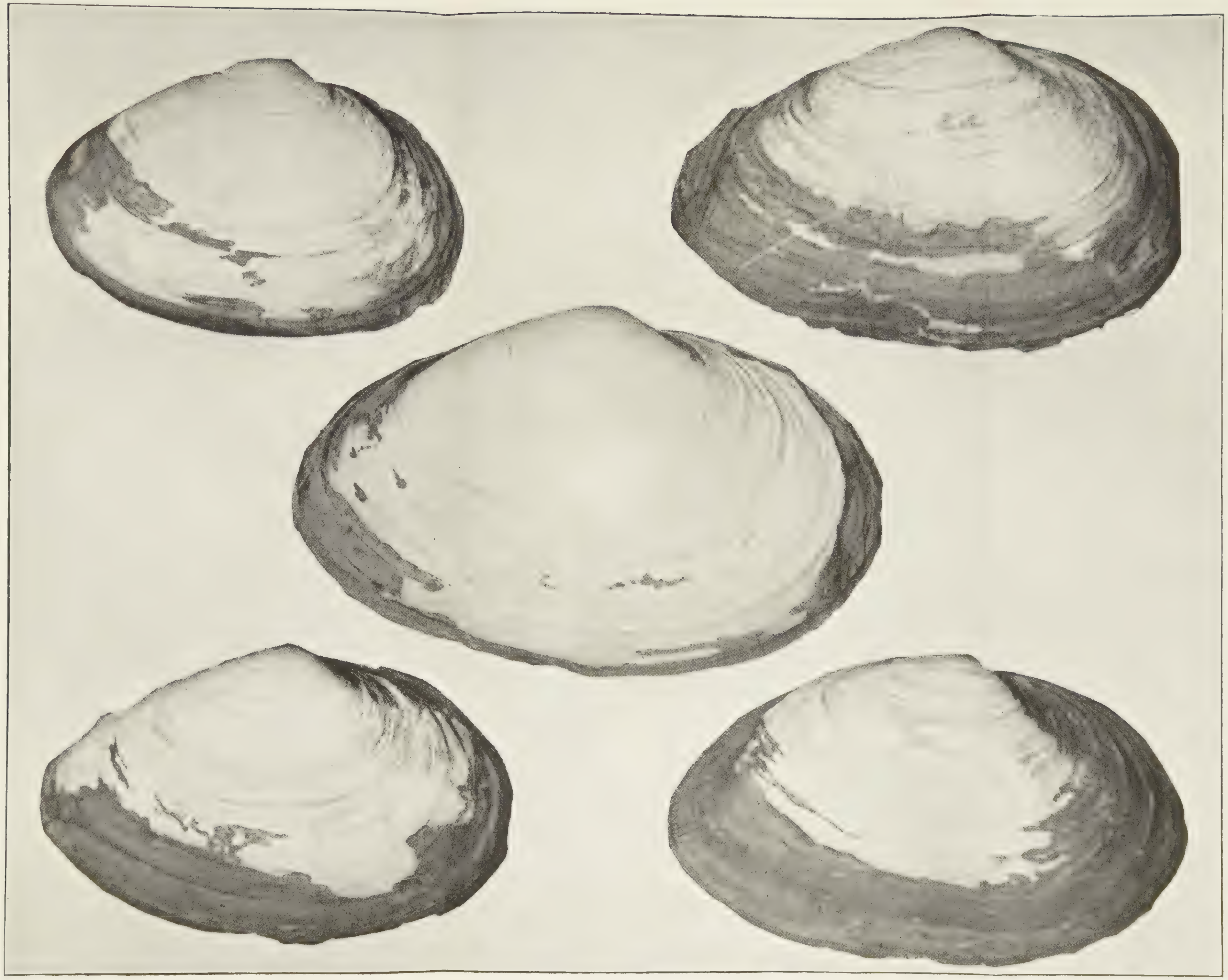


Fig. 4.—Sample clams from the Commission's clam bed at Kickemuit river.

were found to have more than doubled their length, and in some instances to have trebled it.

Experiment No. 4. On July 22, 1899, a number of clams from the same shore were planted separately in land tiles set perpendicularly in the ground, and arranged in rows, from extreme low-water mark to high water mark, at intervals of about two feet. A part of those in one row were measured again about two months later, on September 18th; they showed a decided difference in the rate of growth, those planted at low tide having grown very much more rapidly than those higher up. In the lowest tiles (except the first, which was empty) they measured respectively 48, 46, and 44mm. (1⅞, 1¹³⁄₁₆, 1¹¹⁄₁₆ inches) in length—nearly two inches for clams about three months old.

The more rapid growth of clams lying near low-water mark as compared with those higher up on the shore, so well illustrated here, has often been observed in other instances, and is evidently due to the fact that those at low water have a much longer time each day in which to feed.

Experiment No. 5. On August 14, 1900, about 1,300 small clams were taken from an artificial spat collector and transplanted in sand boxes suspended about one foot beneath the surface of the water at the houseboat. About the first of October they were transferred to new boxes on the shore, where they have remained since that time. At the houseboat they grew remarkably fast, but since they have been on the shore the growth has not been above the average. On August 14, the date of transferring to the houseboat, they averaged about 12mm. (½ an inch) in length and, on September 20, they had grown to three times this length and averaged 36mm. (1½ inches).

This case furnished a beautiful example of the extreme variations in growth, inasmuch as the clams which were left in the spat collector were also measured on September 30 and had increased by only half their length, that is 5mm. (⅕ of an inch). Compare Figure No. 3, Plate No. 2, report for 1900.

At the end of the first year the transplanted specimens averaged about 50 mm. (2 inches), at the end of the second year 74mm. (3 inches) and, when three years and four months old, 80mm. (3⅛ inches). The growth in the third year was probably rather slower than that of average clams under favorable conditions, for the conditions were, in this case, not particularly favorable.

The plotted curve, diagram II, represents the average size of these specimens during three and one-half years. Fig. 13 is from a life size photograph of the average specimens taken at intervals during this time.

Experiment No. 6. More than 20 bushels of very small clams, averaging about 10,000 to the quart, were taken from the shore of Green's Island in the summer of 1901 and transplanted in several of the commission beds at various places. The growth of these transplanted clams, and of those which remained at Green's Island, was observed from time to time.

At the end of six months sample specimens were taken from Buttonwoods and from Rumstick. They exhibited a very noticeable difference in their growth, those from Rumstick averaging 37.5mm. (1½ inches), while those at Buttonwoods averaged 50.2mm., a trifle over 2 inches.

In January, 1903, when the clams were about a year and a half old, those transplanted at Cornelius Island, Wickford, averaged 58.5mm. (2 5-16 inches); those at Mill Cove, Wickford, 63.mm. (2 7-16 inches); those at Kickemuit River, 68.1mm. (2⅝ inches); and those left at Green's Island, 57.4mm. (2¼ inches).

When examined in the latter part of 1903, when the clams were two and a third years old, those at Mill Cove averaged 85.3mm. (3⅜ inches), those at Kickemuit River, 91mm. (3⅝ inches); and those which remained at Green's Island, 76mm. (3 inches).

The average length of these clams in all the experiments was for the first half year 41.8mm. (1⅝ inches); for the first year and a half, 61.7mm. (2 7-16 inches); and for two and a third years, 84mm. (3⅜ inches).

Experiment No. 7. The question is often raised—what is the effect of close planting or overcrowding on the rate of growth? We are, as yet, not prepared to give a final answer, for the evidence from different observations has been more or less contradictory. The clams planted in the sand boxes at the houseboat, described under experiment No. 2, grew very rapidly, though they were so close together that their shells almost touched. On the other hand, the following experiment showed a very distinct dwarfing effect from overcrowding.

In September, 1902, a pint and a half of very small clams which remained over from another experiment were poured upon the ground and covered with a circular sieve to keep them from becoming scattered. The clams were spread as evenly as possible, but were so thick that they touched one another, and in some places were piled two or three deep. Just beside this place other clams of the same size were planted about two inches apart and covered with wire screening. The next day all of the clams, with the exception of a few crushed ones, had burrowed, and, after the sieve was taken up, the circular area was plainly defined by the abundance of clam holes. The clams were, from this time, protected by wire screening and left undisturbed for about one year. When the netting was removed and the place examined again, in October, 1903, the outline of the original area of thickly planted clams could still be traced readily, on account of the numerous holes, although it had become somewhat uneven. The clams were small and stubby, and the shells were thick and deeply scarred. Although they were now about a year and four months old, they were not as large as the average clams of six months.

The control specimens, which were planted near them and given plenty of room, showed at this time a good average growth. In Fig. 14 are shown samples of both kinds.

Experiment No. 8. In this experiment we made use of the device mentioned on page 54, of recording the growth by notching the shell. Clams were selected from various localities of different sizes and ages. After each specimen was carefully notched, they were all planted at one time, on February 20, 1903, in the same locality, on the shore of Mill Cove, Wickford. The specimens were divided into three classes, according to their age and size, as follows:

1. Clams taken form Nayatt Point averaging 25mm. (1 inch), and undoubtedly of the set of 1902, age about seven months.

2. Clams taken from Green's Island, averaging 60mm. ($2\frac{1}{4}$ inches), undoubtedly of the set of 1901, age about one year and seven months.

3. Clams taken from the Kickemuit River at one of the low winter tides. The time of setting is unknown, but the clams showed, by their large size and thick shells, that they were of great age.

At various dates during the next summer samples of these clams (about 25 at a time) were taken up, measured, and preserved. The notch and its growth ring on each specimen prove an accurate record of the size of the clam at the beginning of the experiment. The average measurement of these samples from the 20th of February, 1903 (the beginning of the experiment), and on the dates next examined, together with the percentage in increase, is given below:

DATE OF OBSERVATION.	SET OF 1902.			SET OF 1901.			VERY OLD CLAMS.		
	mm.	inches	Percentage increase in length.	mm.	inches.	Percentage of increase.	mm.	inches.	Percentage of increase.
Feb. 20.........	25	1	......	59.00	$2\frac{5}{16}$	......	106.0	$4\frac{1}{8}$	......
July 6.........	40	$1\frac{9}{16}$	60	65.00	$2\frac{9}{16}$	10	106.0	$4\frac{1}{8}$	0.0
Aug. 10.........	43	$1\frac{5}{8}$	72	68.00	$2\frac{10}{16}$	15	106.0	$4\frac{1}{8}$	0.0
Sept. 10.........	47	$1\frac{7}{8}$	88	70.00	$2\frac{3}{4}$	18	106.0	$4\frac{1}{8}$	0.0
Oct. 20.........	61	$2\frac{3}{8}$	144	71.80	$2\frac{13}{16}$	21	106.3	$4\frac{3}{16}$	0.3

The clams were further grouped, according to size, into six classes regardless of age, and the result of this classification is as follows:

AVERAGE MEASUREMENTS.

SELECTED SIZES.	JULY 6.			AUGUST 10.		
	Average original size.	Average size.		Average original size.	Average size.	
	mm.	*mm.*	*inches.*	*mm.*	*mm.*	*inches.*
Over 3 inches	76.0	78.6	$3\frac{1}{8}$	77.0	79.6	$3\frac{1}{8}$
Between 2½ and 3 inches	66.0	70.2	$2\frac{13}{16}$	67.0	72.1	$2\frac{7}{8}$
Between 2 and 2½ inches	54.0	60.0	$2\frac{3}{8}$	57.2	66.0	$2\frac{3}{8}$
Between 1½ and 2 inches	45.0	58.0	$2\frac{5}{16}$	43.2	60.8	$2\frac{3}{8}$
Between 1 and 1½ inches	30.2	47.2	$1\frac{7}{8}$	29.4	48.5	$1\frac{7}{8}$
Between ½ and 1 inch	21.1	35.0	$1\frac{3}{8}$	21.2	37.2	$1\frac{1}{2}$

AVERAGE MEASUREMENTS.

SELECTED SIZES.	SEPTEMBER 10.			OCTOBER 20.		
	Average original size.	Average size.		Average original size.	Average size.	
	mm.	*mm.*	*inches.*	*mm.*	*mm.*	*inches.*
Over 3 inches	79.0	82.0	$3\frac{1}{4}$	77.0	82.0	$3\frac{1}{4}$
Between 2½ and 3 inches	66.8	73.9	$2\frac{15}{16}$	68.7	76.5	$3\frac{1}{8}$
Between 2 and 2½ inches	53.8	65.0	$2\frac{9}{16}$	53.2	70.6	$2\frac{13}{16}$
Between 1½ and 2 inches	47.7	63.6	$2\frac{1}{2}$	46.6	69.0	$2\frac{3}{4}$
Between 1 and 1½ inches	28.1	56.0	$2\frac{1}{4}$	28.0	64.8	$2\frac{9}{16}$
Between ½ and 1 inch	21.8	47.0	$1\frac{7}{8}$	21.8	59.3	$2\frac{3}{8}$

Statistical Statements of the Age and Size according to Experiments and Observations on the rate of Growth of Clams up to the beginning of the year 1904.

Experiment Number.	Age.	Length.		Time under observation.	Percentage of increase in length while under observation.	Percentage of increase in length per week since the last previous observation.	
		mm.	Inches.			Number of weeks since the previous observation.	Weekly Percentage.
No. 1.	4 weeks.	6.1	$\frac{1}{4}$	....	....	......	....
	6 "	9.5	$\frac{3}{8}$	10 days.	$55\frac{3}{4}$ pr. ct.	1.4	$36\frac{3}{4}$ pr. ct.
	7 "	9.9	$\frac{3}{8}$	17 "	62 "	1.0	$4\frac{1}{5}$ "
	9 "	13.9	$\frac{1}{2}$	31 "	127 "	2.0	$20\frac{1}{5}$ "
	$12\frac{1}{2}$ "	17.5	$\frac{5}{8}$	56 "	186 "	3.4	$7\frac{3}{7}$ "
	14 "	18.8	$\frac{11}{16}$	65 "	208 "	1.2	7 "
	17 "	23.7	$\frac{7}{8}$	88 "	278 "	3.2	$7\frac{3}{5}$ "
	27 "	27.7	$1\frac{1}{16}$	153 "	354 "	9.2	$1\frac{7}{10}$ "
No. 2.	4 "	6.1	$\frac{1}{4}$	....	....	......	....
	8 "	21.8	$1\frac{3}{16}$	28 days.	257 pr. ct.	4.0	64 pr. ct.
	12 "	23.2	$\frac{7}{8}$	53 "	280 "	3.5	$1\frac{1}{2}$ "
	14 "	25.6	1	63 "	319 "	1.4	5 "
	17 "	29.0	$1\frac{1}{8}$	85 "	377 "	3.1	6 "
No. 5.	2 mos.	12.1	$\frac{7}{16}$	....	....	......	....
	3 "	16.9	$\frac{5}{8}$	$3\frac{1}{2}$ weeks.	23 pr. ct.	3.5	$6\frac{4}{7}$ pr. ct.
	$3\frac{1}{2}$ "	36.0	$1\frac{7}{16}$	$5\frac{1}{4}$ "	197 "	1.75	$30\frac{1}{3}$ "
	$4\frac{1}{4}$ "	40.0	$1\frac{9}{16}$	9 "	230 "	3.75	0.5 "
	1 yr. 2 "	50.0	2	1 yr. 7 days.	313 "	43.0	0.5 "
	1 " 7 "	68.0	$2\frac{11}{16}$	1 " $5\frac{1}{4}$ mos.	462 "	21.0	1.7 "
	2 " 2 "	74.0	$2\frac{15}{16}$	2 "	511 "	29.0	0.3 "
	3 " 4 "	80.0	$3\frac{1}{8}$	3 " 2 mos.	561 "	60.0	0.1 "
No. 6.	2 "	9.5	$\frac{3}{8}$	....	....	......	....
	7 "	50.2	2	5 mos. 8 days	427 pr. ct.	22.0	19.4 pr. ct.
	7 "	37.5	$1\frac{1}{2}$	5 " "	294 "	22.0	16.1 "
	1 yr. 7 "	57.4	$2\frac{1}{4}$	1 yr. 5 mos.	504 "	73.0	6.9 "
	1 " 7 "	58.5	$2\frac{5}{16}$	1 " 5 "	515 "	73.0	7. "
	1 " 7 "	63.0	$2\frac{7}{16}$	1 " 5 "	563 "	73.0	7.7 "
	1 " 7 "	68.0	$2\frac{5}{8}$	1 " 5 "	610 "	73.0	8.3 "
	2 " 6 "	76.0	3	2 yrs. 4 "	700 "	47.0	0.7 "
	2 " 4 "	85.3	$3\frac{3}{8}$	2 " 2 "	798 "	39.0	0.8 "
	2 " 4 "	91.0	$3\frac{5}{8}$	2 " 2 "	858 "	39.0	0.8 "
	7 "	25.0	1	....	....	......	....
	1 "	40.0	$1\frac{9}{16}$	$4\frac{1}{2}$ mos.	65 pr. ct.	20.0	3.2 pr. ct.
	1 " 1 mo.	43.0	$1\frac{11}{16}$	$5\frac{1}{2}$ "	70 "	4.5	1.6 "
	1 " 2 "	47.5	$1\frac{3}{4}$	$6\frac{1}{2}$ "	109 "	4.5	2.3 "
	1 " 3 "	65.2	$2\frac{9}{16}$	$7\frac{1}{2}$ "	150 "	4.5	3.9 "
	1 " 7 "	60.16	$2\frac{3}{8}$	....	....	......	....
	2 "	66.01	$2\frac{9}{16}$	$4\frac{1}{2}$ mos.	10 "	20.0	.5 pr. ct.
	2 " 1 mo.	68.0	$2\frac{5}{8}$	$5\frac{1}{2}$ "	15 "	4.5	.7 "
	2 " 2 "	69.5	$2\frac{3}{4}$	$6\frac{1}{2}$ "	17 "	4.5	.4 "
	2 " 3 "	71.6	$2\frac{3}{4}$	$7\frac{1}{2}$ "	20 "	4.5	.6 "
	1 " 7 "	60.0	$2\frac{3}{8}$	1 yr. 5 "	533 "	73.0	7.3 "

Statistical Statements of the Age and Size according to Experiments and Observations on the rate of Growth of Clams up to the beginning of the year 1904.

Experiment Number.	Age.	Length.		Time under observation.	Percentage of increase in length while under observation.	Percentage of increase in length per week since the last previous observation.	
		mm.	Inches.			Number of weeks since the previous observation.	Weekly Percentage.
No. 6.	1 yr. 7 mos.	62.0	$2\frac{1}{2}$	1 yr. 5 mos.	554 pr. ct.	73.0	7.6 pr. ct.
	1 " 7 "	64.0	$2\frac{9}{16}$	1 " 5 "	575 "	73.0	7.8 "
	1 " 7 "	62.0	$2\frac{1}{2}$	1 " 5 "	554 "	73.0	7.6 "
	1 " 7 "	68.1	$2\frac{3}{4}$	1 " 5 "	624 "	73.0	8.5 "
	1 " 7 "	59.5	$2\frac{3}{8}$	1 " 5 "	533 "	73.0	7.3 "
	1 " 7 "	62.5	$2\frac{1}{2}$	1 " 5 "	554 "	73.0	7.6 "
	1 " 7 "	63.0	$2\frac{1}{2}$	1 " 5 "	570 "	73.0	7.7 "

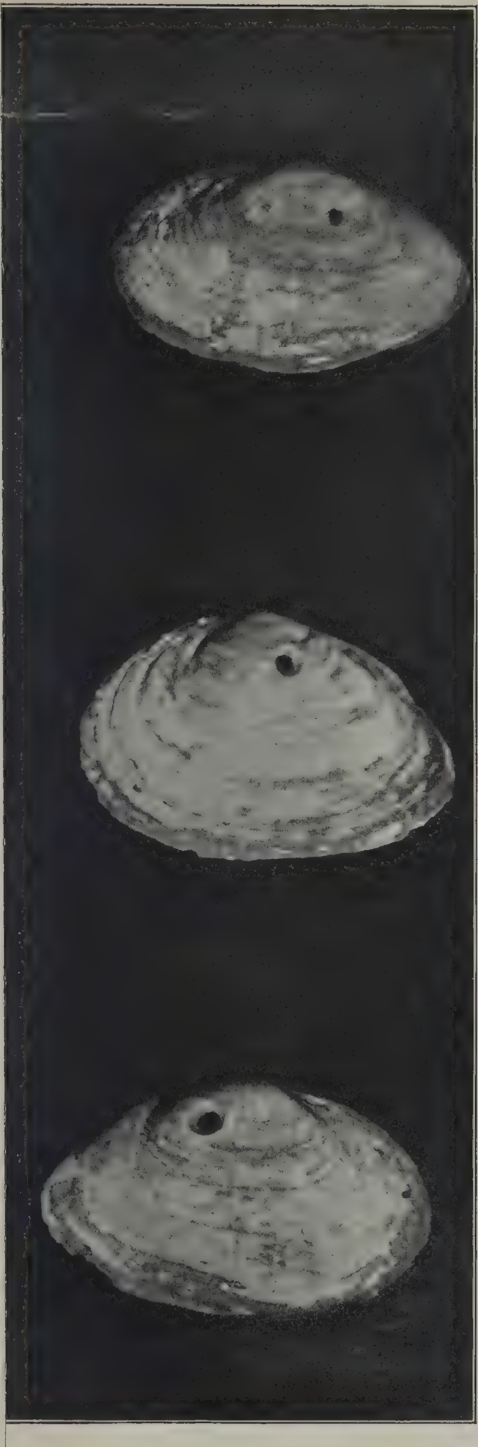
RESULTS OF EXPERIMENTS IN TRANSPLANTING.

The experiments we have just been discussing have demonstrated that clams of any age and size can be transplanted successfully, and that the rate of growth under favorable conditions justifies the expectation of a crop in about two years from the date of spawning. We have been able, also, to test the feasibility of transplanting by experiments on a comparatively large scale.

In accordance with the act passed by the General Assembly, at its January session, in the year 1901, the commission was enabled to occupy and have the exclusive right in certain areas of shore, between high and low water, set aside for experimental purposes (see Appendix). Taking advantage of this opportunity, experiments were made in transplanting the clams found in such phenomenal abundance at Green's Island in the summer of 1901.

The clams were taken when they were very small and transplanted to different localities in different parts of the Bay on the reservation beds. The data in respect to the planting, and the results of the experiments as shown by examination of the beds in January, 1903, a year and a half after planting, are tabulated as follows:

Number.	Location.	Area.	Amount sowed.	Aver. No. per qt.	When sowed.	Condition of Tide.	Condition of wind.	Time between digging and sowing.	How kept.	Remarks.
1	*S. shore, Mill Cove*	*a* *50 x 25 ft.*	*36 qts.*	*2,135*	*Aug. 14, 11 a. m.*	*Just turning..... Low about 12.*	*None.*	*24 hours.*	*In water..*	*1-3 unplanted.*
	Very poor soil. At time of planting and for sometime before there were *no* clams in plot. Now a very few, not over 2 or 3 per square foot. None at all in the third unplanted.									
1		*b* *50 x 25 ft.*	*54 qts.*	*2,135*	*Aug. 17. 5:30 p. m.*	*Coming in....... Low about 2:30.*	*None.*	*26 hours.*	*Dry......*	*Plot partly grass.*
	Three diggings gave an average of 17 per square foot. (1) Area 15 x 15 inches produced 16 clams. (2) Area 15 x 15 inches produced 28 clams. (3) Area 1 x 1 foot produced 21 clams. The average length 60 mm. (2⅜ inches).									
2	*S. shore, Cornelius Island*..............	*a* *50 x 25 ft.*	*36 qts.*	*2,135*	*Aug. 14, 1 p. m.*	*Coming in....... Low about 12.*	*None.*	*24 hours.*	*In water..*	*Loamy sand.*
	Upper half of bed gave 24 per square foot. Area 15 x 15 inches produced 37 clams. Lower half (miry sand) 7 to 8 per square foot. Average length 62 mm. (2⅛ inches).									
2		*b* *50 x 25 ft*	*54 qts.*	*2,135*	*Aug. 16, 5:30 p. m.*	*Half in .... Low about 1:40.*	*None.*	*26 hours.*	*In water..*	*Loamy sand.*
	Upper half produced 34 per square foot. Area 15 x 15 inches produced 51 clams. Lower half (miry sand) 9 to 11 per square foot. Average length 64 mm. (2 9-16) inches).									
3	*Rumstick*	*150 x 50 ft.*	*72 qts.*	*9,700*	*Aug. 17, 3 p. m.*	*Coming in....... Low about 2:40.*	*Brisk.*	*2 hours.*	*Dry.......*	*Fine gravel.*
	Only occasionally a clam.									
4	*Fishing Cove (Wickford)*...............	*a* *25 x 50 ft.*	*45 qts.*	*9,700*	*Aug. 18, 10 a. m.*	*High .... Low about 3:30.*	*Light.*	*20 hours.*	*Dry.......*	*Fine gravel.*
	Two diggings averaged 16 per square foot. (1) 15 x 15 inches produced 24 clams. (2) 15 x 15 " " 23 " Average length 62 mm. (2½ inches.)									



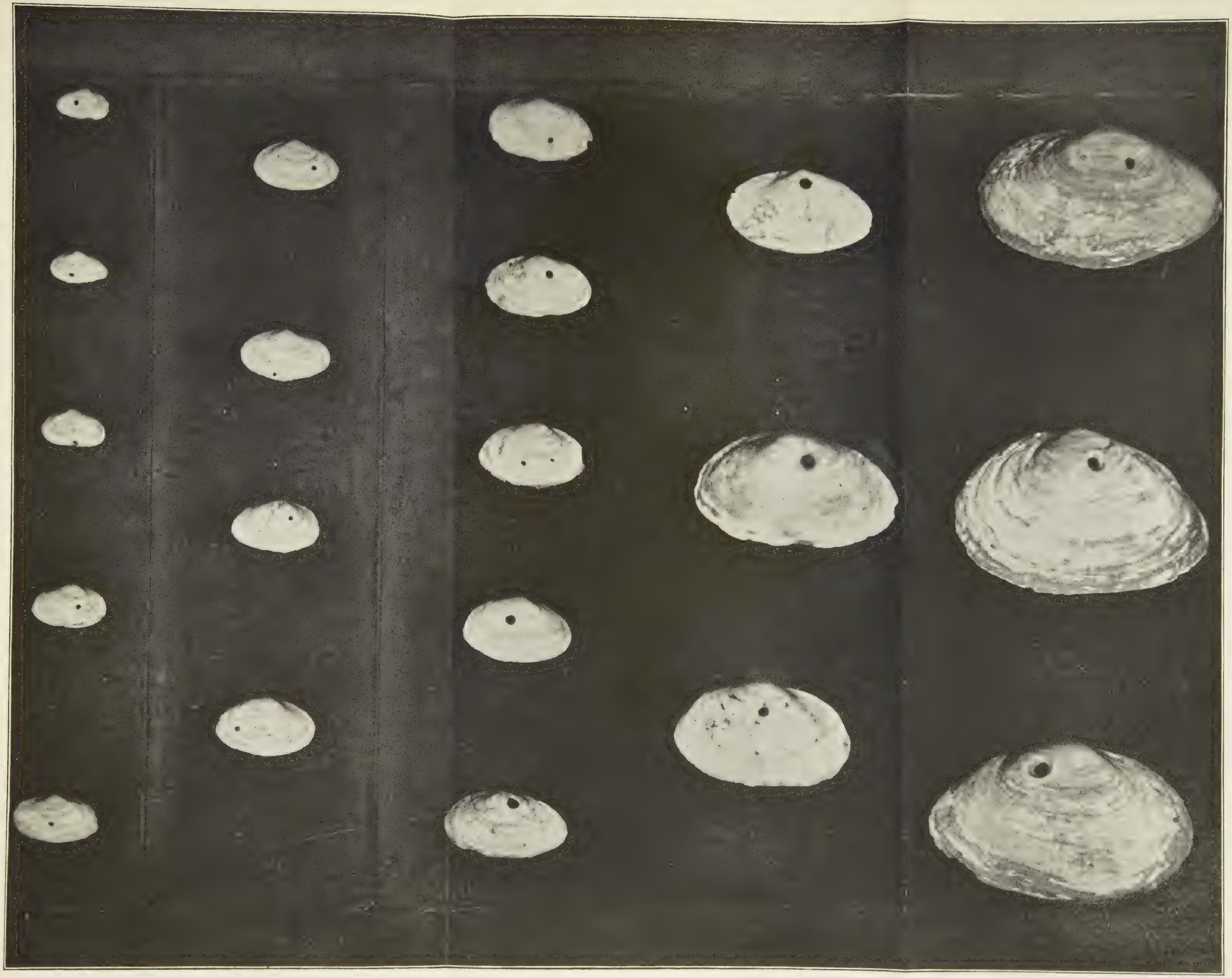


Fig. 7.—Bored clams, showing that clams of all sizes are attacked by the borers.

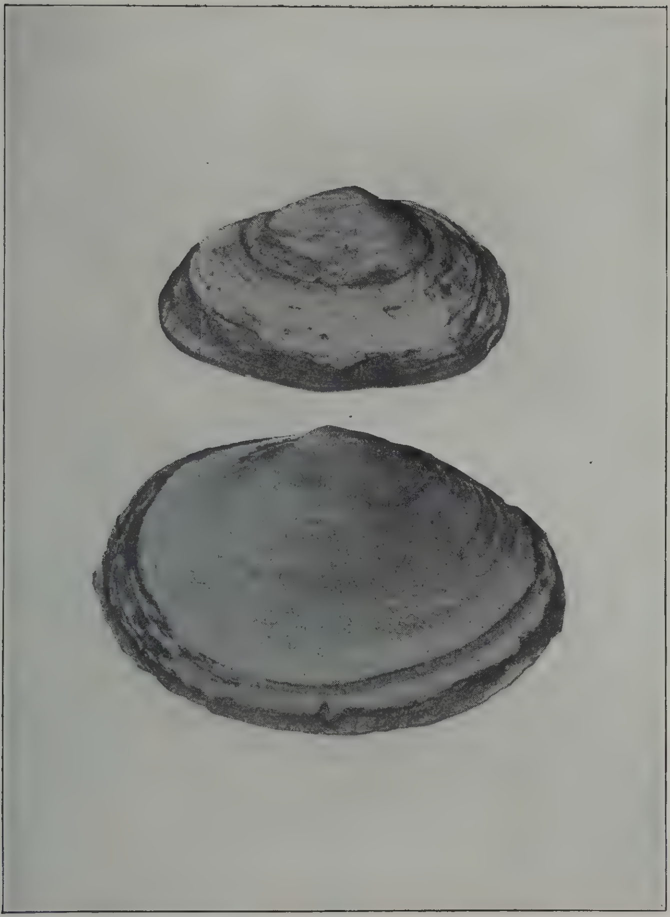


FIG. 8.—Notched clams, showing growth from February 20 to July 6, 1903.
The upper one is from the set of 1902.
The lower one is from the set of 1901,

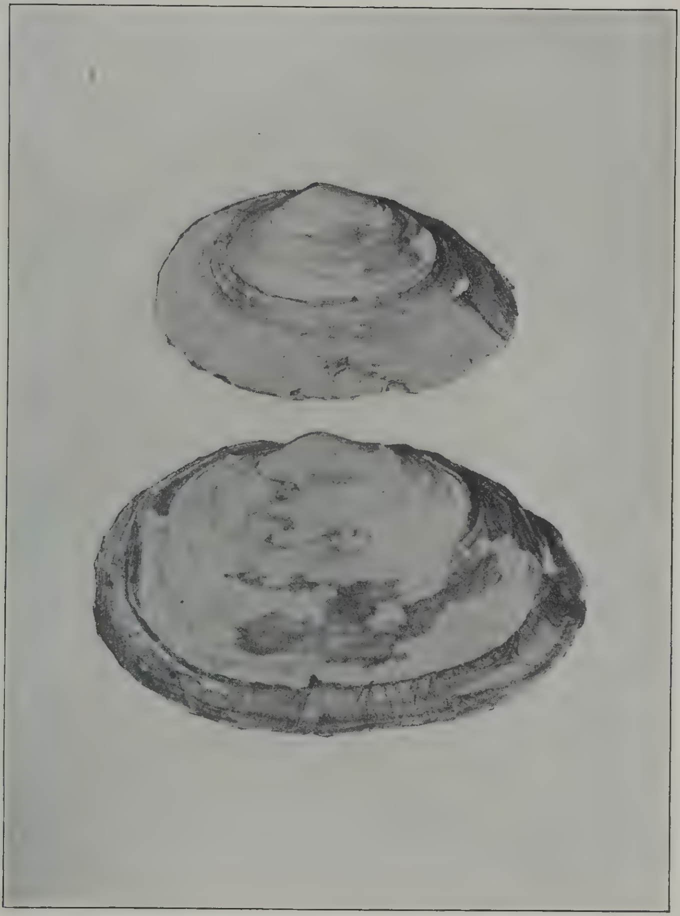


FIG. 9.—Notched clams, showing growth between February 20 and August 10, 1903.
The upper one is from the set of 1902.
The lower one is from the set of 1901.

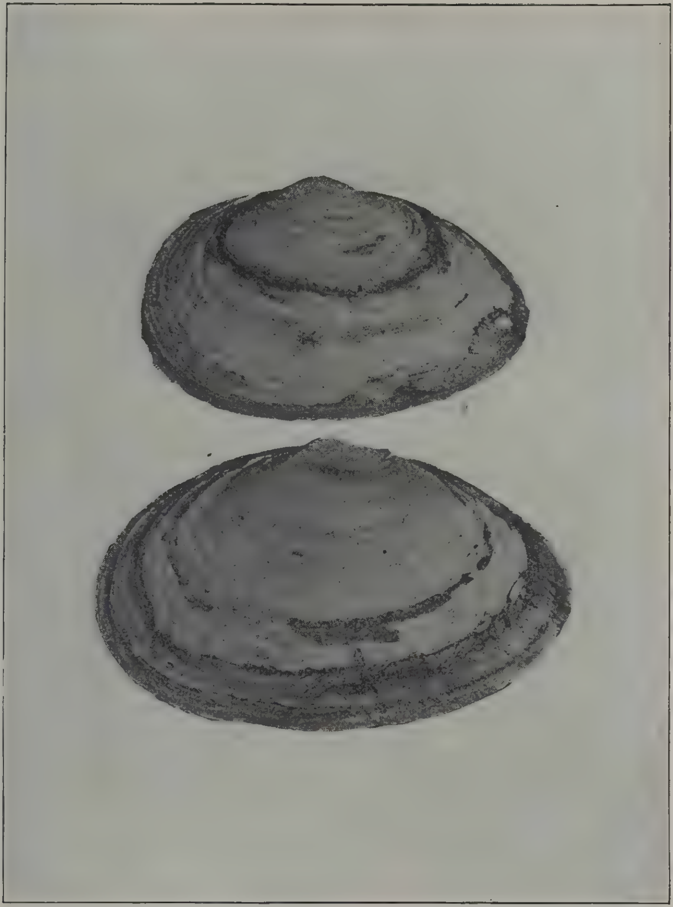


FIG. 10.—Notched clams, showing the growth between February 20 and October 30, 1903.

The upper one is from the set of 1902.

The lower one is from the set of 1901.

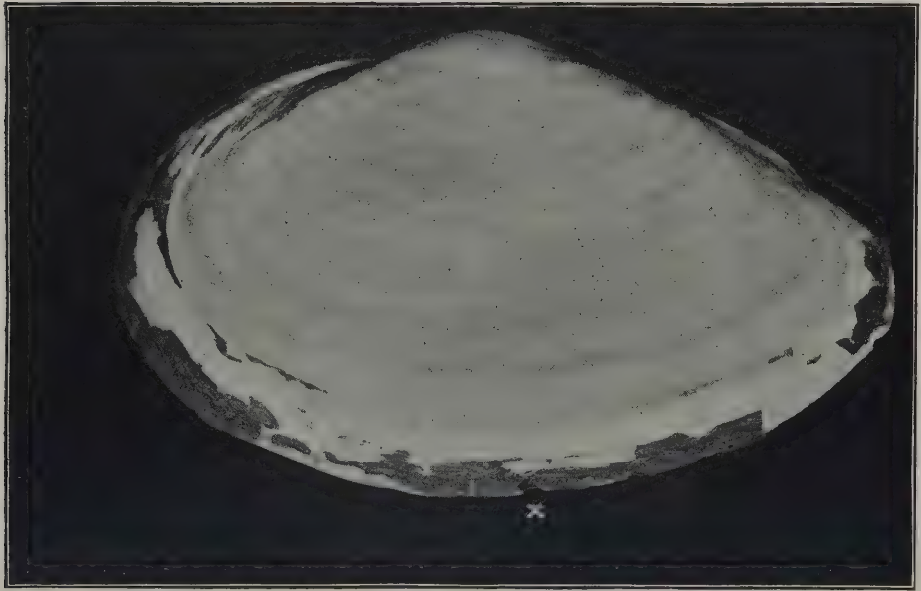


Fig. 11.—A clam of extreme age notched February 20, and taken up October 20, 1903. Shows no growth.

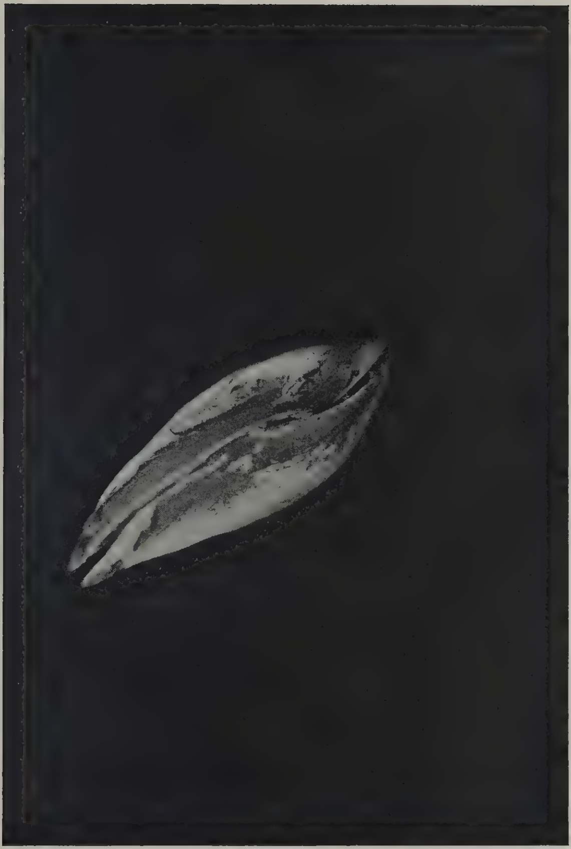


FIG. 11½.—Specimen of clam contorted by developing in stony ground.

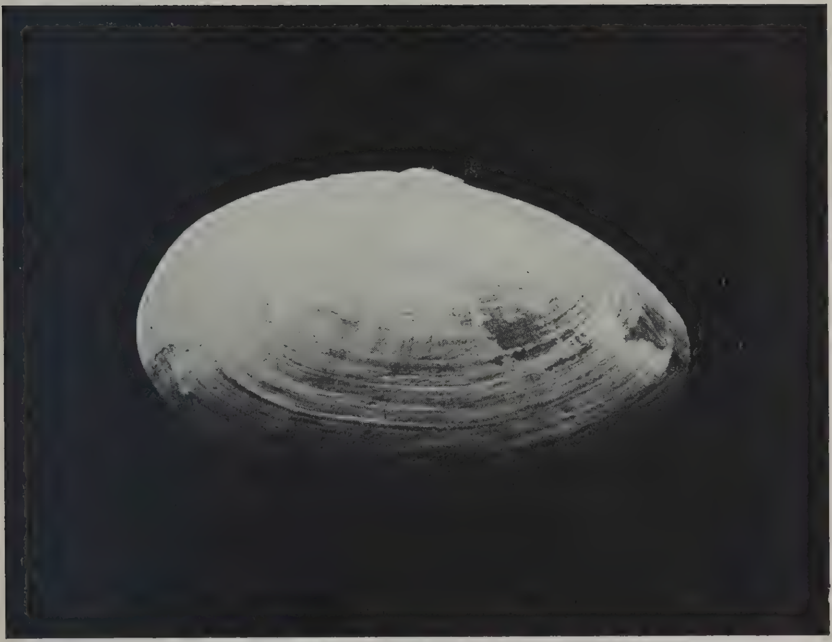
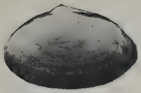
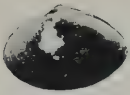


Fig. 12.—One of the land-tile specimens planted July 18, 1899, when of the size of the smaller specimen in Fig. 5, and dug on September 10, 1900. It is, therefore, about a year and three months old.

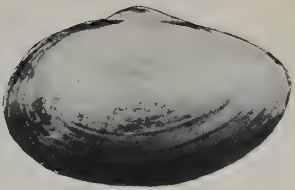
Aug. 14, 1900.



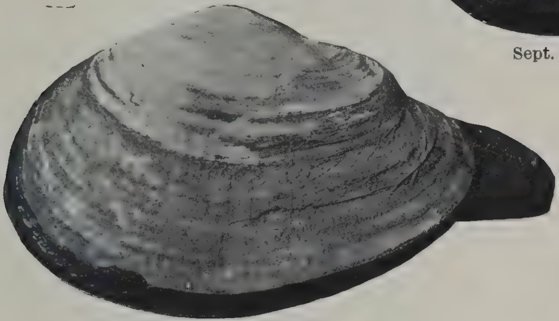
Sept. 8, 1900.



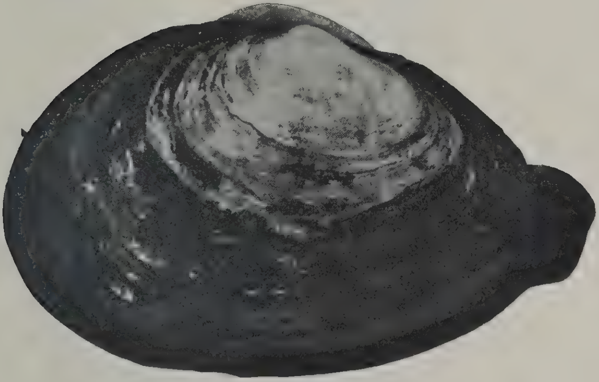
Sept. 30, 1900.



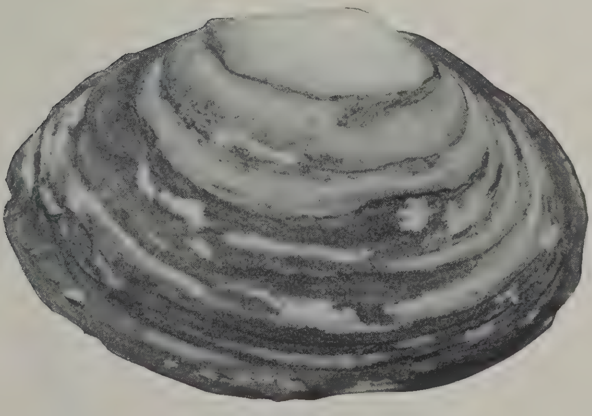
Sept. 20, 1900.



Jan. 30, 1902.

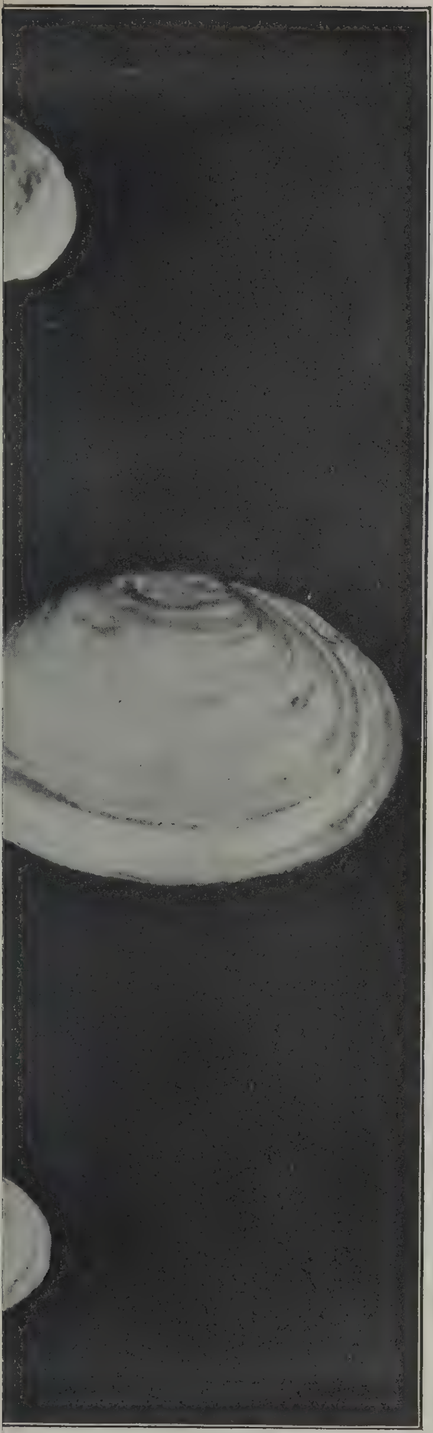


Jan. 17, 1903.



Oct. 20, 1903.

Fig. 13.—Clams set in the summer of 1900 in clam catcher, and raised in sand boxes. The various specimens, taken at intervals, represent their growth during three years and four months. The difference in size of the two specimens on Sept. 20, 1900, is due to the difference in locality.



All the clams are of the same age

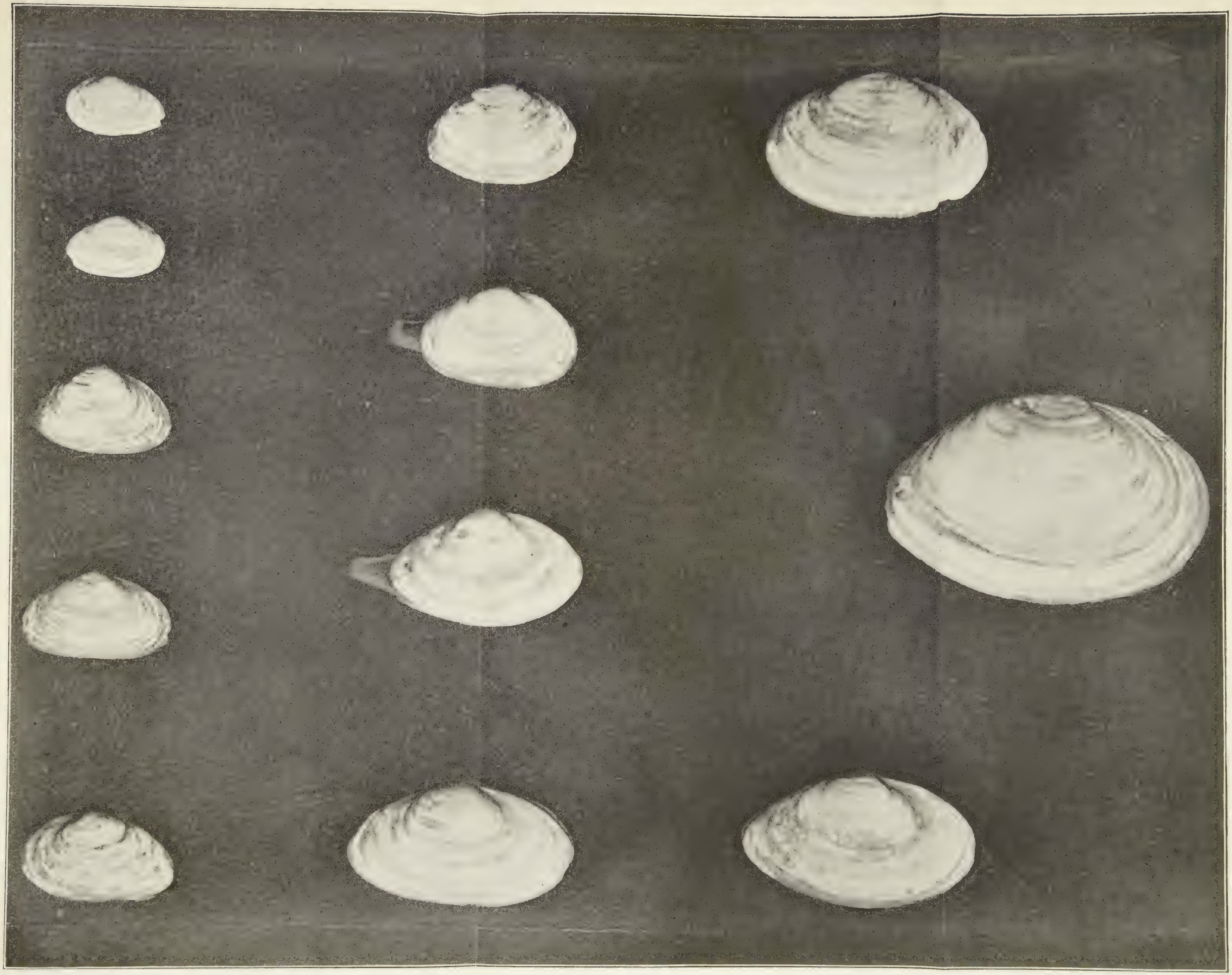
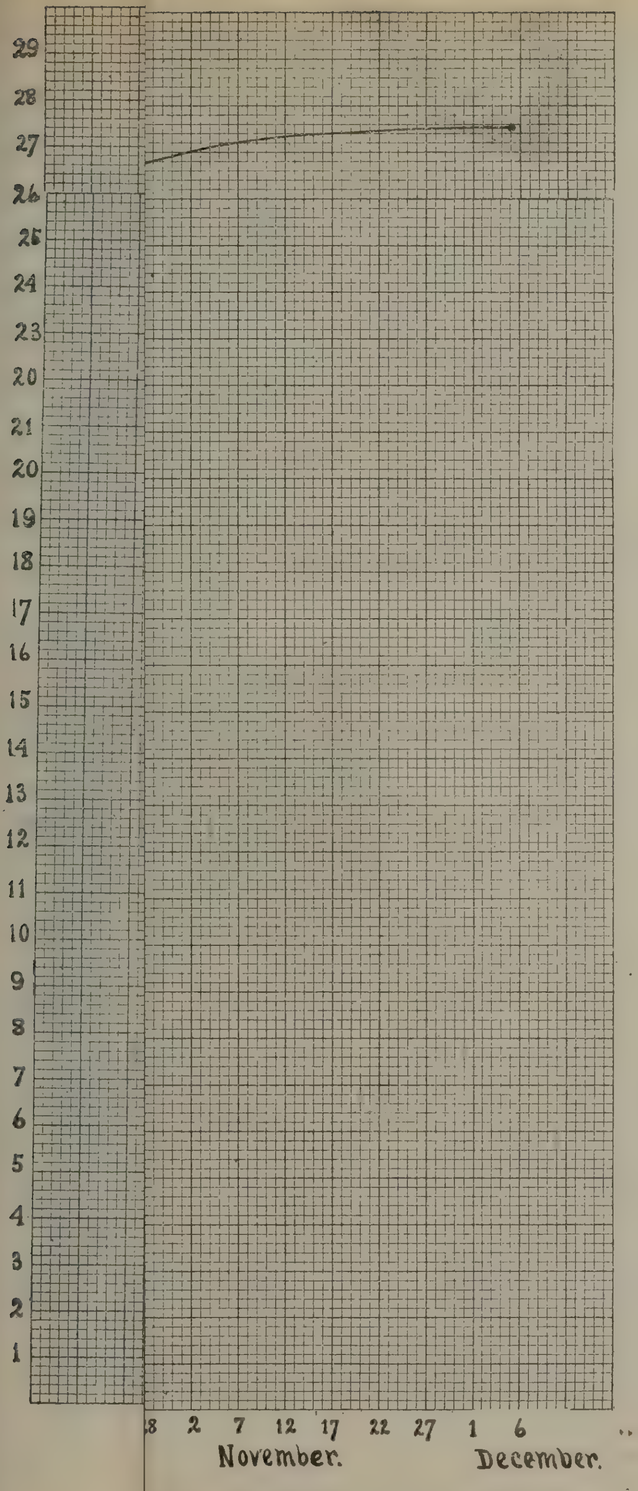


FIG. 14.—Stunted clams, showing the effect of excessive crowding. The large clam on the right is from a control bed, where it had plenty of room. All the clams are of the same age (about one year and four months old).



kept submerged.

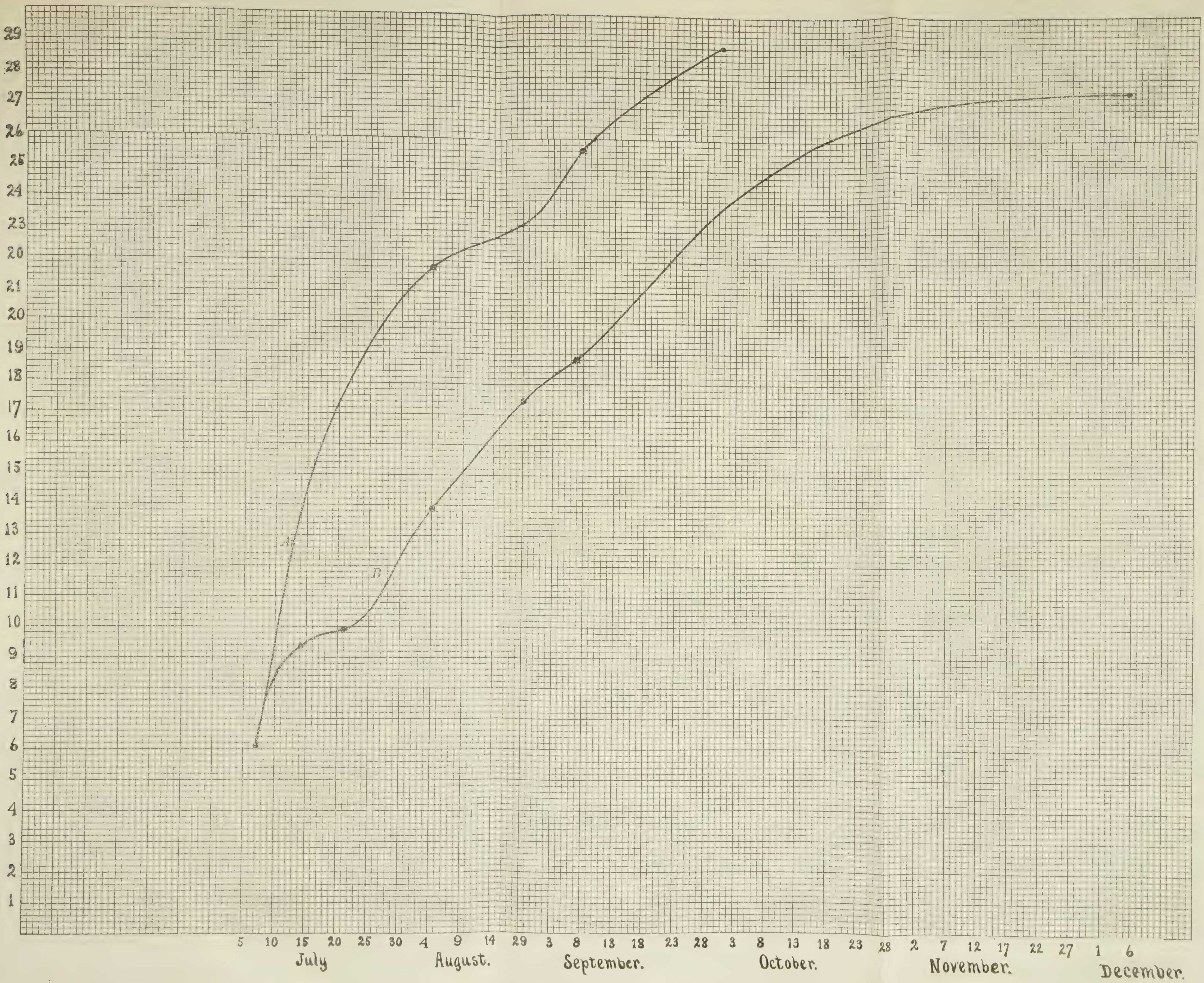
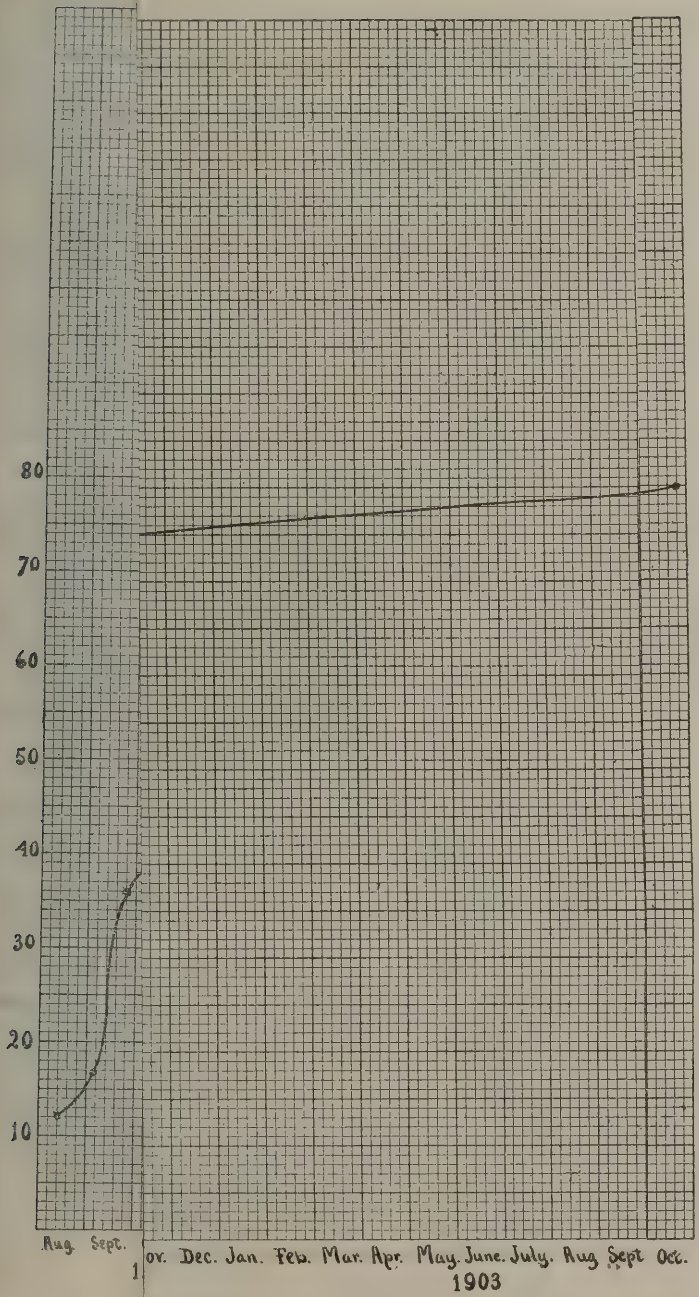


Diagram 1. Represents graphically the difference in growth of clams exposed by the tide and those kept submerged.
Curve A. Clams submerged at the House Boat. (See Experiment No 2, p. 56.)
Curve B. Clams exposed by the tide at Cornelius Island. (See Experiment No. 1, p. 55.)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nt No. 5, p. 57.)

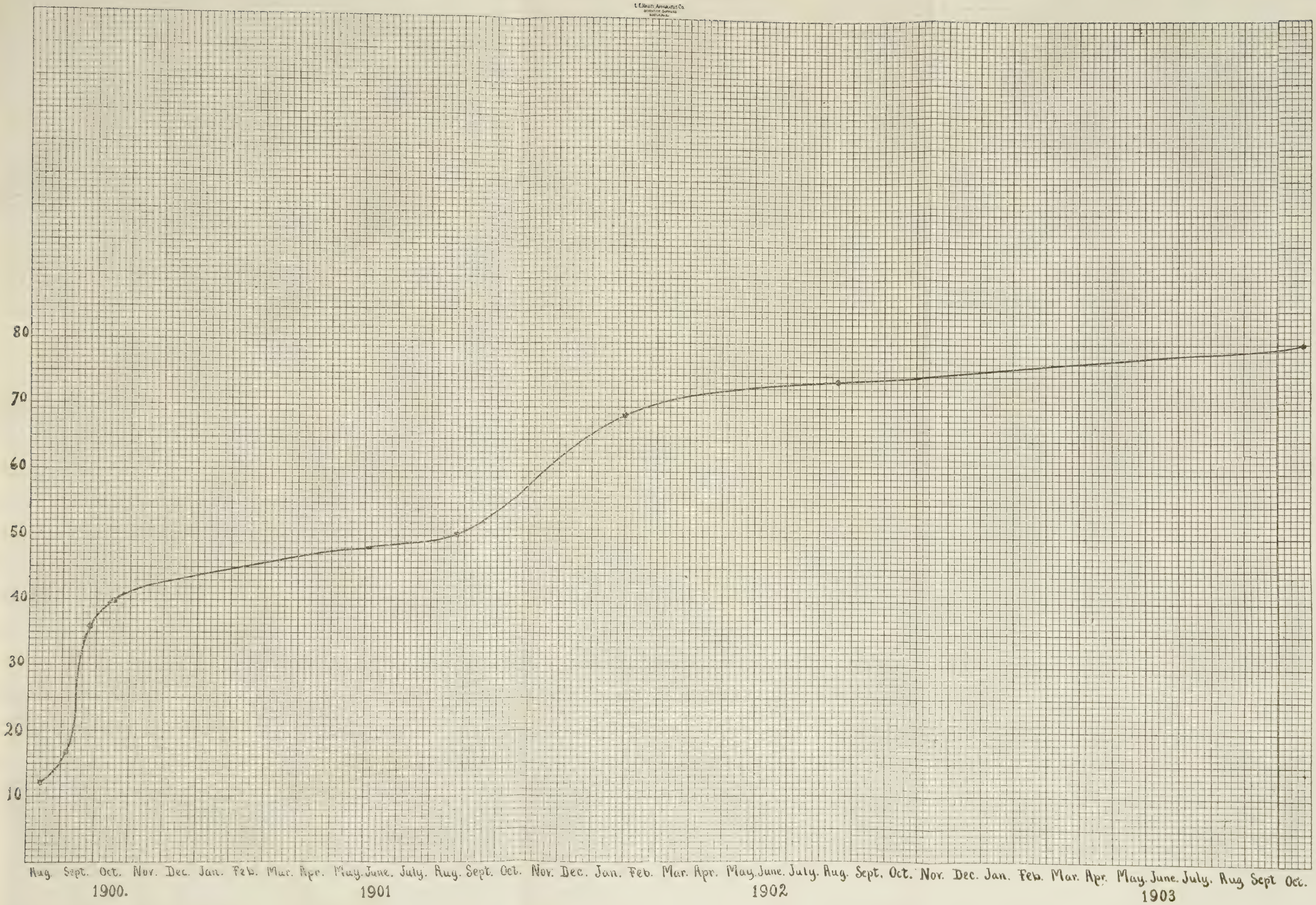
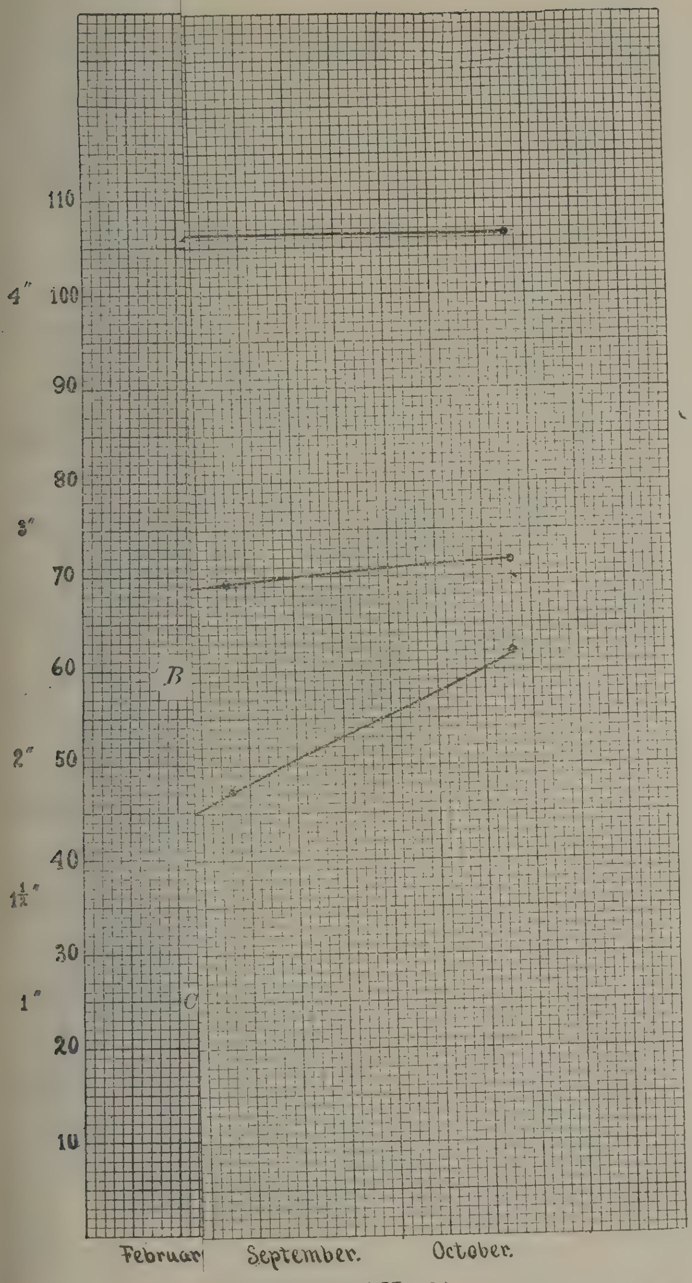


Diagram II. Plotted curve of growth of clams of the "artificial set." (See Experiment No. 5, p. 57.)



See Experiment No. 8.)

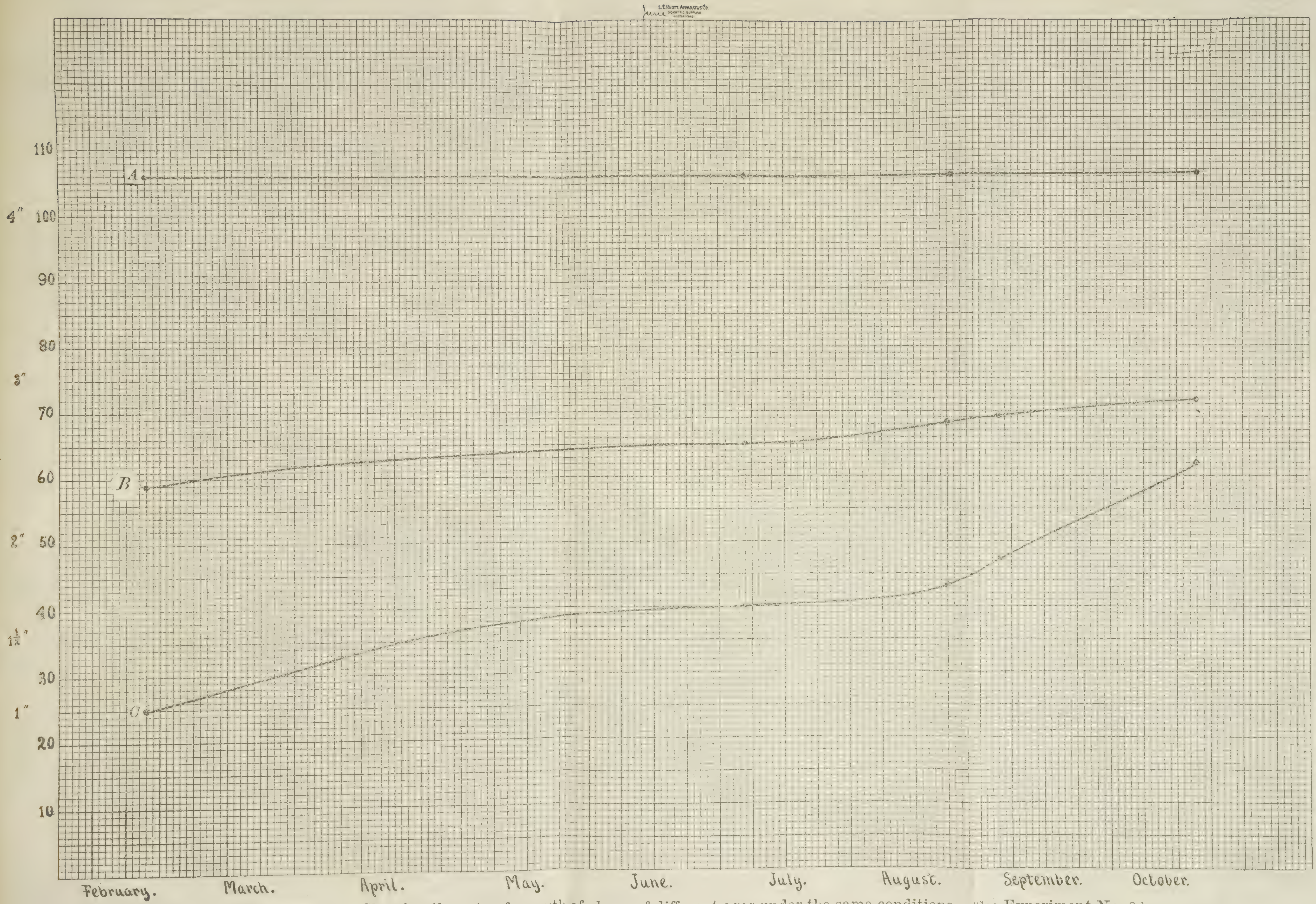
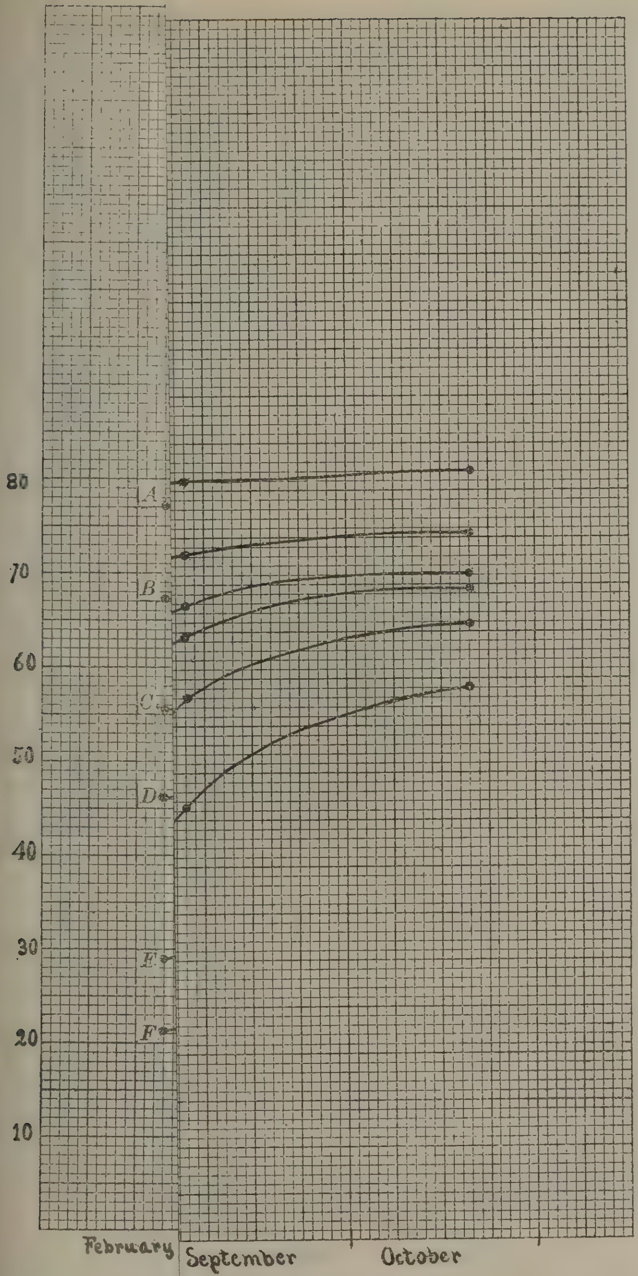


Diagram III. Showing the rate of growth of clams of different ages under the same conditions. (See Experiment No. 8.)

A. Clams of extreme age.
B. Clams from the set of 1901.
C. Clams from the set of 1902.



eriment No. 8.)

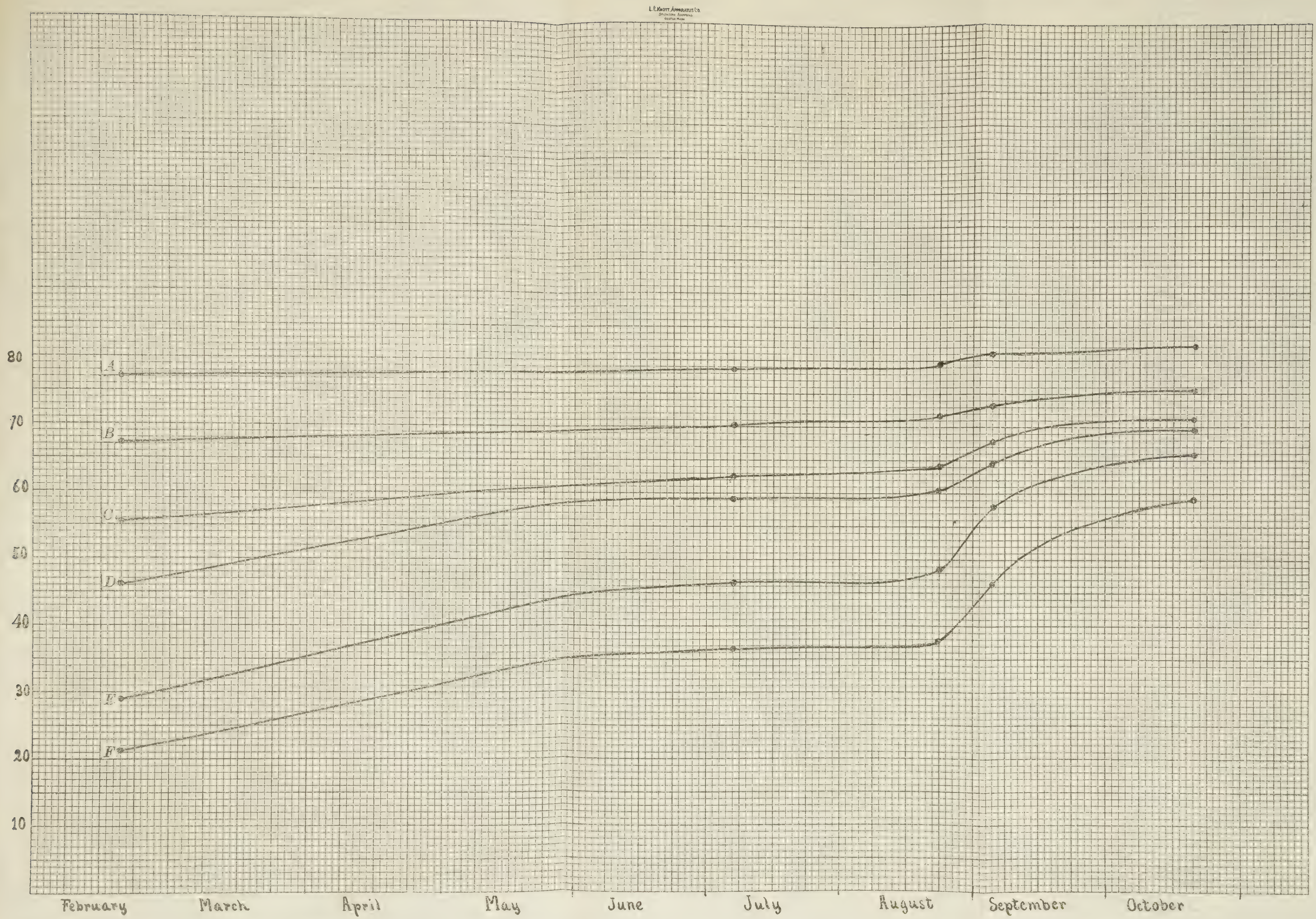


Diagram IV. Showing the rate of growth of selected sizes of clams, regardless of age. (See Experiment No. 8.)

A. Clams 3 inches in length and over.
B. Clams from 2½ to 3 inches in length.
C. Clams from 2 to 2½ inches in length.
D. Clams from 1½ to 2 inches in length.
E. Clams from 1 to 1½ inches in length.
F. Clams under 1 inch in length.

Number.	Location.	Area.	Amount sowed.	Aver. No. per qt.	When sowed.	Condition of Tide.	Condition of wind.	Time between digging and sowing.	How kept.	Remarks.
4	One digging gave 14 per square foot. 15 x 15 inches produced 21 clams.	b 25 x 50 ft.	54 qts.	9,700	Aug. 24, 5 p. m	Half out........ Low about 7:20.	Light.	4 1-2 hours	Dry......	Fine gravel.
5	*Kickemuit River* .... One digging in a place with average number of holes gave 34 per square foot. 15 x 15 inches produced 127 clams or 3 quarts. (Some of them of more recent set)	a 60 x 150 ft.	108 qts.	9,700	Aug. 19, 2 p. m.	Going out........ Low about 4:10.	Light.	4 1-2 hours	Dry......	Sandy loam.
5	One digging gave 90 per square foot 15 x 15 inches produced 138 clams or 4 quarts. (Some of more recent set.) Average length 68 mm. (2¾ inches).	b 60 x 150 ft.	108 qts.	9,700	Sept. 7, 9:30 a. m	Low. .... Low about 8.....	None.	24 hours.	Dry......	Sandy loam.
6	*Smith's Pasture, Mill Cove*............. Two diggings gave average 23 clams per square foot. 18 x 18 inches produced 56 clams. 18 x 18 " " 51 " As on Cornelius (2 (a) and (b)) lower edge of bed clams very poor. Average length 59.5 mm. (2⅜ inches).	200 x 60 ft.	153 qts.	9,700	Aug. 23, 3 p. m.	Going out .... Low about 6.	Light.	4 hours.	Dry......	Marl.
7	*Duck Cove (Wickford)*.................. Very few present. Average length 62.5 mm. (2½ inches).	100 x 50 ft.	45 qts.	9,700	Aug. 24, 6 p. m.	Going out........ Low about 7.	Light.	4 hours	Dry......	Rocky beach.
8	*South shore, Mill Cove* .... One digging produced 32 per square foot. 15 x 15 inches produced 46 clams. Average length 63 mm. (2½ inches).	100 x 60 ft.	108 qts.	9,700	Aug. 26, 5:30 p. m	High tide........ Low about 10.	Light.	4 hours	Dry......	Coarse gravel &

Number.	Location.	Area.	Amount sowed.	Aver. No. per qt.	When sowed.	Condition of Tide.	Condition of wind.	Time between digging and sowing.	How kept.	Remarks.
9	*Old Buttonwoods*........................ Showed excellent growth in January, 1901. Since then clams have disappeared; either many been dug or otherwise destroyed.	*150 x 60 ft.*	*99 qts.*	*9.700*	*Aug. 28, 1 p. m*	*Half in*........... *Low about 10.*	*Light.*	*3 hours.*	*Dry*.......	*Shifting sand.*
10	*Rabbitt Island* (*Wickford*).............. Produced a great quantity of clams. Bed was dug over many times before clams were 1 year old.	*100 x 50 ft.*	*54 qts.*	*9,700*	*Aug. 28, 4 p. m.*	*Almost in*........ *Low about 11:40.*	*Light.*	*6 hours.*	*Dry*.......	*Fine gravel and sand.*
11	*N. shore, Cornelius Island*............. Poor locality; no results.	*200 x 20 ft.*	*45 qts.*	*9,700*	*Aug. 29, 5 p. m.*	*High*............. *Low about 12:30.*	*Light.*	*3 hours.*	*Dry.*	*Coarse gravel.*
12	*Quonset*............................... Produced good quantity. Thoroughly dug up.	*60 x 60 ft.*	*36 qts.*	*9,700*	*Sept. 5, 10 a. m.*	*Half in*.......... *In, 1:20.*	*Light.*	*20 hours.*	*Dry*.......	*Sandy and shifting.*
13	*Cold Spring* (*Wickford*)............... Evidence of some digging; no results.	*60 x 60 ft.*	*40 qts.*	*9,700*	*Sept. 5. 11 a. m.*	*Coming in*....... *In, 1:20.*	*Light.*	*21 hours.*	*Dry.*	*Sandy gravel.*

It will be seen by consulting the tables that, in the greater number of beds, the clams were thriving well and were promising an excellent crop at the end of 18 months. Nearly all of the experiments were spoiled, however, before the clams became two years old, by poachers. It is practically impossible to stand guard night and day over so many beds. The experiments, though they were interrupted, nevertheless show that it is feasible to raise clams by this method, also that the greatest difficulty is to provide protection until the crop can mature.

One of the experiments, that in the Kickemuit River, was carried to completion, and the clams were left practically undisturbed for two years.

We are glad to acknowledge our special obligation to the members of the firm of C. E. Brown & Sons, and to Mr. Charles Hull, through whose aid it was possible to complete, for the first time, an experiment in transplanting clams on a large scale in Narragansett Bay.

On July 20th, 17 bushels of clams were dug from a space about 300 square feet, by two men, at one tide, and they had finished digging within an hour after the tide had begun to rise.*

At this rate of production the yield would be more than 2,400 bushels per acre. From one square yard three pecks of clams were dug, corresponding to a yield of 3,600 bushels per acre.

The bed was opened to the public on August 10th, 1903, and shortly after midnight the diggers began to assemble and made use of the early morning tide, which was low at about two o'clock. At low tide in the afternoon, more than 50 persons were engaged in digging upon this bed. The ground was continually dug over throughout the rest of the summer, and even as late as November. On October 20, for example, there were eleven persons digging on this bed.

According to the estimate of those who have watched the yield from this bed most closely, about 600 to 700 bushels have been taken from it The bed is about $\frac{2}{5}$ of an acre in extent. This estimate is low as compared with the proportionate yield of the sample areas already referred to.

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* Probably a considerable number of clams were overlooked in so rapid digging, which would, of course, raise the production from this area.

In some parts of the bed there were evidences that the clams were killed at an early age, perhaps by the decaying seaweed which was very abundant, and some dead specimens also were found near the entrance of a small fresh water stream.

A large set of young clams was found in this bed, and, in some sample areas, they numbered three hundred to the square yard. They were of different sizes and had, of course, set since the bed was sown in the summer of 1901.

This experiment affords the final proof of two fundamental propositions, viz., 1st, that it is feasible, practicable, and profitable to transplant clams, and that a good crop can be reared in two years; 2nd, that it is not necessary or profitable to dig up the shore and loosen the soil in order that clams may grow and thrive well, or in order that they may set thickly.

THE DISTRIBUTION OF SEWAGE IN NARRAGANSETT BAY, WITH ESPECIAL REFERENCE TO THE CONTAMINATION OF THE OYSTER BEDS.

By C. A. FULLER.

Appendix to the Report of the Commissioner of Fisheries to the Secretary of Commerce and Labor for the year ending June 30, 1904.
A Thesis submitted to the Faculty of Brown University
for the degree of Doctor of Philosophy.

DEPARTMENT OF COMMERCE AND LABOR
BUREAU OF FISHERIES
GEORGE M. BOWERS, Commissioner

THE DISTRIBUTION OF SEWAGE IN THE WATERS OF NARRAGANSETT BAY, WITH ESPECIAL REFERENCE TO THE CONTAMINATION OF THE OYSTER BEDS

BY

CALEB ALLEN FULLER
Assistant in Wisconsin State Hygienic Laboratory

APPENDIX TO THE REPORT OF THE COMMISSIONER OF FISHERIES TO THE SECRETARY OF COMMERCE AND LABOR FOR THE YEAR ENDING JUNE 30, 1904
Pages 189 to 238

WASHINGTON
GOVERNMENT PRINTING OFFICE
1905

DEPARTMENT OF COMMERCE AND LABOR
BUREAU OF FISHERIES
GEORGE M. BOWERS, Commissioner

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By CALEB ALLEN FULLER
Assistant in Wisconsin State Hygienic Laboratory

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THE DISTRIBUTION OF SEWAGE IN THE WATERS OF NARRAGANSETT BAY, WITH ESPECIAL REFERENCE TO THE CONTAMINATION OF THE OYSTER BEDS.[a]

By Caleb Allen Fuller,
Assistant in Wisconsin State Hygienic Laboratory.

INTRODUCTION AND REVIEW OF LITERATURE ON "OYSTER INFECTION."

More than twenty years ago attention was called to the fact that oysters and other shellfish which are eaten raw might be the cause of some of the outbreaks of typhoid fever and cholera which have occurred from time to time in certain coast towns of England and Ireland. Among the first to support this view strongly was Sir Charles Cameron. After examining some oyster beds on the northern shore of Dublin Bay, he suggested that "oysters taken from this source were quite as likely to be a source of typhoid infection as milk or water." He found these oyster beds in a most unhealthy condition. The oysters were sick and died in large numbers every year. Investigation of the beds showed them to be "literally bathed in sewage," and the oysters were found to contain sewage matters within the shells. In 1880 he read before the British Medical Association a paper entitled "Oysters and typhoid," in which he called attention to the fact that contaminated oysters might be the cause of these outbreaks of typhoid fever and cholera in the coast towns of England and Ireland.

No special interest was manifested in this statement until, in 1893, Doctor Thorne-Thorne, in his report to the local government board for that year, gave it as his opinion that certain sporadic cases of cholera which had occurred at various inland places in England in that year were due to oysters and other shellfish from sewage-contaminated water at Grimsby, where there had been a small outbreak of the disease. Following out Doctor Thorne-Thorne's suggestion, the Government commenced an exhaustive series of investigations, the results of which have appeared in the annual reports of the local government board. This work was carried out under the direction of Doctors Bulstrode and Klein.

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[a] Thesis submitted to the faculty of Brown University for the degree of Doctor of Philosophy.

In brief, the results of their experiments are the following: "The oyster does not, under normal conditions, contain, either within its body or in the liquor inclosed by its shell, any microbe than can grow in phenolated gelatin or in phenol broth." Three species of bacteria were isolated from normal oysters. "A minute motile bacillus, capable of liquefying gelatin very rapidly," is "by far the most abundant micro-organism in the ordinary oyster." "Occcasionally only, a spore-forming, motile bacillus is also obtained, which corresponds culturally to *B. vulgatus.* A nonliquefying bacillus is, too, as a rule, present, which differs culturally from *B. coli* in the circumstance that it will not grow in broth at 37° C. The number of microbes of the above sorts present in the liquor and in the body of the oyster varies greatly in different samples; of oysters from the same batch, some afford few, some innumerable colonies to the gelatin-plate culture test. Having satisfied myself in the above sense that bacteria of excremental origin are not, in the ordinary course, apt to be contained within oysters, I set myself to ascertain whether *B. coli* and *B. typhosus* were not discoverable in oysters * * * from sources under more or less suspicion of sewage contamination."

The media used for these tests were phenol broth and gelatin. The results of the examination show that "Oysters from a few out of numerous batches derived from sources where they did appear to be exposed to risk of sewage contamination were found to exhibit colon bacilli. In one case where the circumstances were especially suspicious, Eberth's typhoid bacillus was found in the mingled body and liquor of the oyster." Though Doctor Klein regards the presence of colon organisms in oysters as an indication of sewage contamination, he was not able to show a constant relation between sewage contamination of the water and the presence of these organisms in shellfish. Certain batches of oysters from apparently polluted waters were found to contain *B. coli*, while other lots from apparently equally polluted sources did not give positive reactions for this bacillus.

In 1894 was published the report of Doctor Conn's careful investigation of the famous outbreak of typhoid fever, which occurred at Wesleyan University in October of that year. The account of this epidemic is familiar to all, and only the main facts of the case will be referred to at this time. On October 12 seven college fraternities had their initiation ceremonies and celebrated in the usual way with a supper. Eight days after several students were reported sick, with a moderate degree of fever, and shortly after November 1 twenty-three cases of typhoid fever had developed. Investigation proved beyond a shadow of doubt that the water supply was above suspicion and that the sanitary condition of the boarding and lodging houses was perfect. All the men affected were members of three fraternities which had obtained their oysters from a local dealer. One other fraternity had oysters from the same dealer, but these were eaten cooked, while the other three lots were consumed raw. Two of the remaining three fraternities did not have oysters, and the other one obtained its supply from a dealer in Hartford. Only one non-fraternity man contracted the disease, and the investigation of his case only established more firmly the responsibility of the local supply, for this man had eaten of the same lot of oysters at the dealer's shop. Inquiry brought out the fact that two of five men from Yale who attended the exercises of the societies were seized with typhoid fever some time after their return to New Haven. Further investigation showed that the infected oysters had been stored at the mouth of the Quinnipiac River, 300 feet from the outlet of a small drain from a house in which two persons lay sick with typhoid fever.

In 1894, Doctor Casey reported in the British Medical Journal a case of fatal "oyster poisoning," and since that date the pages of this publication contain frequent references to the subject of "oyster infection."

In 1895, Sir William Broadbent published the facts of a series of cases and groups of cases of typhoid fever and other gastro-intestinal illnesses, which he concludes were caused by the ingestion of raw oysters. There was no bacteriological evidence that the oysters were polluted, but circumstances pointed strongly to these shellfish as the cause of the disease. The following case is typical of those reported. Sir William was called to see a young woman who, ten to fourteen days previous, had eaten some raw oysters in company with a cousin. She developed a mild case of typhoid, as did also the cousin, who had gone to Italy. Another similar case: A clergyman and his daughter, living in the country where typhoid was unknown, were seized with this disease. Inquiry revealed the fact that they had eaten raw oysters in London while on a visit to that city some two weeks previous.

In the same year Sir Peter Eade emphasized the fact that mussels and other shellfish, as well as oysters, might become a source of infection. Doctor Wilson reported three instances, occurring in Florence, where persons who had eaten raw oysters were taken sick with typhoid fever, while other persons in the same parties who did not eat oysters were not ill. A little later Doctor Johnson-Lavis reported some cases of typhoid and gastro-intestinal disorders of a very severe type which he encountered in his practice in Naples in 1879. These illnesses were most prevalent among strangers who had eaten raw oysters. Investigation showed that oysters were brought to Naples from seacoast towns, where there was no typhoid, and stored for a long time in the harbor in a bed less than 60 feet distant from the outlet of one of the main sewers. These oysters were filled with sewage matters, and "when they were consumed about a tablespoonful of sewage water was swallowed." He is positive that these oysters are responsible for the prevalence of these diseases in Naples at that time.

In 1896 Doctor Chantemesse reports a number of cases of typhoid in a village where there had been no cases of that nature for over a year. Fourteen persons in the village ate a lot of oysters from Cette and were made sick. Others of the same families who had not eaten of this lot suffered no inconvenience. Eight of the fourteen were slightly ill and four others very severely ill, with diarrhea and intestinal disturbances. The two remaining persons developed very severe cases of typhoid fever, one of which terminated fatally. Bacteriological examination of oysters from several localities demonstrated the presence of *B. coli* in large numbers.

Doctor Mosny, who has made a most careful and thorough investigation of the whole subject of mollusk poisoning in France, reports a case of "oyster infection" in a village near Paris in 1900. Five members of a family of seven were stricken with severe gastro-intestinal disturbances after eating some oysters from Cette.

Many other similar cases are reported in the French medical literature, the conclusions, however, all based on circumstantial evidence.

In 1900 Doctor Plowright reported a number of cases of enteric fever, due to contaminated clams. In the village of North Lynn (entire population 70) 30 persons ate clams on several occasions in May and June. Of the 30 who ate the clams, 15 consumed them raw and 10 of the 15 came down with typhoid fever. None of those who ate them cooked experienced any trouble. The "clams were dug in a mud flat at the mouth of the Great Ouse, 3 miles below the point at which the town of King's Lynn discharges its untreated sewage. * * * Similar cases of enteric fever following the consumption of uncooked clams have simultaneously been observed in the town itself and in other surrounding villages."

In 1900 the Philadelphia Medical Journal published the account of several cases of typhoid occurring at Portland, Me. Four of an Italian crew came down with typhoid some time after eating mussels which they had gathered from the piles beneath a wharf near which is the outlet of one of the city sewers.

In 1902 Dr. J. C. Thresh published in the Lancet of December 6 the account of 21 cases of typhoid and gastro-intestinal disturbances, which he ascribes to the consumption of raw oysters. His account includes the histories of six families in which the illnesses occurred only among persons who had eaten oysters. The cases ranged in severity from one fatal case of typhoid to slight intestinal disturbances. One instance of special interest is the following: All members of a certain family ate these contaminated oysters, and, with the exception of one person, all were sick. This person, not liking the taste of the oysters, did not swallow any and was not made ill.

Bacteriological examination of oysters from the common source revealed the presence of *B. coli* and *B. enteritidis* Gärtner, but not *B. typhosus.*

In the same year it was reported at a meeting of physicians at Pera, Turkey, that a large percentage of typhoid cases which occurred in Constantinople could be traced to the consumption of oysters from polluted sources. Examination demonstrated the presence of *B. coli* in many and of *B. typhosus* in a few specimens.

Also, in 1902, an extremely large number of typhoid cases was reported in Atlantic City, N. J., during the summer months. A very careful investigation of the sanitary condition of the water supply, the milk and food supply, and of the sewage-disposal system was made by Philip Marvel. He came to the conclusion that the increase in the number of these cases was due, in a great measure, to oysters fattened near the outlet of one of the city sewers.

In November, 1902, occurred also the famous "oyster epidemics" at Winchester and Southampton, England, which were investigated by Doctor Bulstrode and reported to the local government board in May, 1903. At two banquets given by the mayors of these cities 267 guests were present. Shortly after the dinners 118 of the guests were attacked with gastroenteritis, and all of these had eaten raw oysters. Twenty-one cases of typhoid fever, 5 of which were fatal, also developed as a result of eating the oysters.

Doctor Fraser reports an epidemic of typhoid fever at Portsmouth, where 25 persons were attacked with this disease after eating raw oysters.

In 1904 the following facts in regard to typhoid due to infected oysters were published in the fourth report of the commissioners of sewage disposal:

Doctor Nash, health officer at Southard-on-Sea, states that 50 per cent of the cases of enteric fever at that town were due to consumption of shellfish from sources contaminated by sewage. Out of 105 cases of that disease at least 85 bore some connection to polluted shellfish; also that the number of cases occurring at Yarmouth was greatly reduced after the sale of mussels was stopped in that town.

Doctor Newsholme, of Brighton, makes the following statement in regard to the cases of typhoid occurring in that city during the years 1894 to 1902: "There were 643 reported during this period; 158 cases were directly ascribable to the consumption of oysters and 80 to other shellfish. In other words, 37 per cent of the total number of cases is due to polluted shellfish." In the opinion of Doctor Newsholme the extent of the illness attributable to shellfish is probably understated.

Doctor Niven, of Manchester, reports 274 cases of typhoid out of 2,664 occurring in that city during the years 1897 to 1902, inclusive, as due to the consumption of shellfish.

Medical officers of London report that about 8 per cent of the cases occurring in London in 1902 were due to shellfish.

In 1895, stimulated by the interest awakened by the epidemic of typhoid fever at Wesleyan University, which Doctor Conn had shown to be due to infected oysters, Doctor Foote, of Yale, brought out the results of some bacteriological experiments on oysters. Though the aim of this work was to find out the length of time the typhoid bacillus could live in experimentally infected oysters, he states, with reference to the bacteriological content of oysters from presumably (?) uncontaminated sources, that no typhoid-like organisms were found in these specimens; the bacteria present in the juice were nearly all anaërobic micrococci. In another series of experiments he found *B. fluorescens liquefaciens* frequently in plates made from the juice, and in one instance *B. gasoformens.* He tested the stomach content of 9 oysters, and found that 8 were sterile. He isolated in these tests more than 10 varieties of bacteria, many of which were not identified, but none gave the reactions of the colon bacillus.

Doctor Giaxa states, however, that "it is a curious fact that in spite of the many varieties of bacteria found in the surrounding water, only two varieties (although in large numbers) could be detected in the oysters examined."

Chantemesse reports the presence of *B. coli* in many oysters from sewage-contaminated sources; also that oysters placed in water previously infected with typhoid stools for twenty hours contained these "typhoid organisms and *B. coli* in great numbers."

Dr. Cartwright Wood, in his work on the bacteriology of the oyster, did not succeed in finding pathogenic forms in shellfish taken from unpolluted sources. He also states that "all species of bacteria found in the juice are identical with the water bacteria found in the water in which the oysters live."

Sabatier, Duchany, and Petit isolated the following organisms from oysters: *Micrococcus fervidosus*, *M. flavus liquefaciens*, *M. radiatus*, *Bacillus fluorescens liquefaciens*, *B. mesentericus vulgatus*, *Streptothrix færsteri*, and *M. luteus.* On the other hand, no colon or typhoid bacilli were found by these observers in oysters "laid down" experimentally within a few feet of the outfall of a large sewer.

Herdman and Boyce, in England, were the next to direct their attention to the problem of oyster infection by sewage. They have shown that the presence of *B. coli* in oysters sold in the markets is by no means an unusual occurrence. In one series of experiments 48 batches of oysters were taken haphazard from the various fish markets of London. From "one-third to one-half of these specimens were found to contain *B. coli*," which was also found in a number of mussels, cockles, and periwinkles examined by them. *B. enteritidis sporogenes* was also found in oysters, mussels, and periwinkles. These observers are of the opinion that the oyster "is more frequently liable to the presence of colon-like organisms than other species of common edible shellfish."

In 1901 Doctor Hill, of the Boston city health department, published the results of the analysis of clams obtained from the Charles River flats, which are exposed to contamination from the Boston sewage. These clams contained *B. coli*, *B. enteritidis sporogenes*, and *B. aerogenes capsulatus.*

In addition to the above list of experiments, a large number of references might be given to scattered outbreaks and sporadic cases of typhoid fever and gastro-enteritis which have been attributed to the ingestion of oysters and other shellfish. In most of these cases, however, no bacteriological examination of the material under suspicion was made, and therefore all evidence is purely circumstantial. For a comprehensive review of this literature the reader is referred to the article by Doctor Harrington, "Some reported cases of typhoid attributed to oysters," published in the Boston Medical and Surgical Journal, Vol. CXLIV, No. 19; to the exhaustive treatise of Doctor Mosny, "Maladies provoquées par l'ingestion des mollusques," in the Revue d'Hygiène, December, 1889, and January, February, and March, 1900; and to an article by Doctor Newsholme, published in the British Medical Journal of August 8, 1903.

Little success has attended the efforts to isolate the typhoid bacillus from contaminated oysters. Doctor Klein found it in but one of a large number of specimens examined. It was also reported in certain oysters from Constantinople. Many experiments have been made, however, to determine the conduct of *B. typhosus* in oysters experimentally inoculated with pure culture, and also to determine the length of time that the typhoid organism and the vibrio of cholera can live in sea water and in oysters and other shellfish. Indeed, much more attention has been given to this phase of the problem than to the bacteriology of normal oysters.

Whatever experiments have been made on normal oysters indicate that the bacterial content is variable, depending more or less on the locality from which the specimens are obtained. Nearly all observers agree that "normal" oysters—that is, oysters living in pure sea water—do not contain *B. coli* or other sewage forms "in their bodies or in the liquor within their shells," and that the bacteria occurring in these specimens are species commonly found in water. There is little doubt but that the germ content of the surrounding water determines, to a great extent, the germ content of oysters and other shellfish living in it. If *B. coli* and other sewage bacteria are present in appreciable numbers in the water we will in all probability find some trace of them in the shellfish. Doctor Houston, however, is of the opinion that *B. coli* is present in many shellfish from a presumably unpolluted source. In regard to the question as to whether the presence of *B. coli* in shellfish can be considered an index of fecal contamination there seems to be considerable difference of opinion. On the one hand Klein asserts this to be the case, and states that "the presence of *B. coli* in the oyster is strongly suggestive of fouling of the particular sample with material of excremental origin," while, on the other hand, Herdman and Boyce are inclined to accept the statement with reserve. From the results of the experiments recorded in this paper, however, it seems to me that the presence of this organism in oysters is a certain indication of sewage contamination.

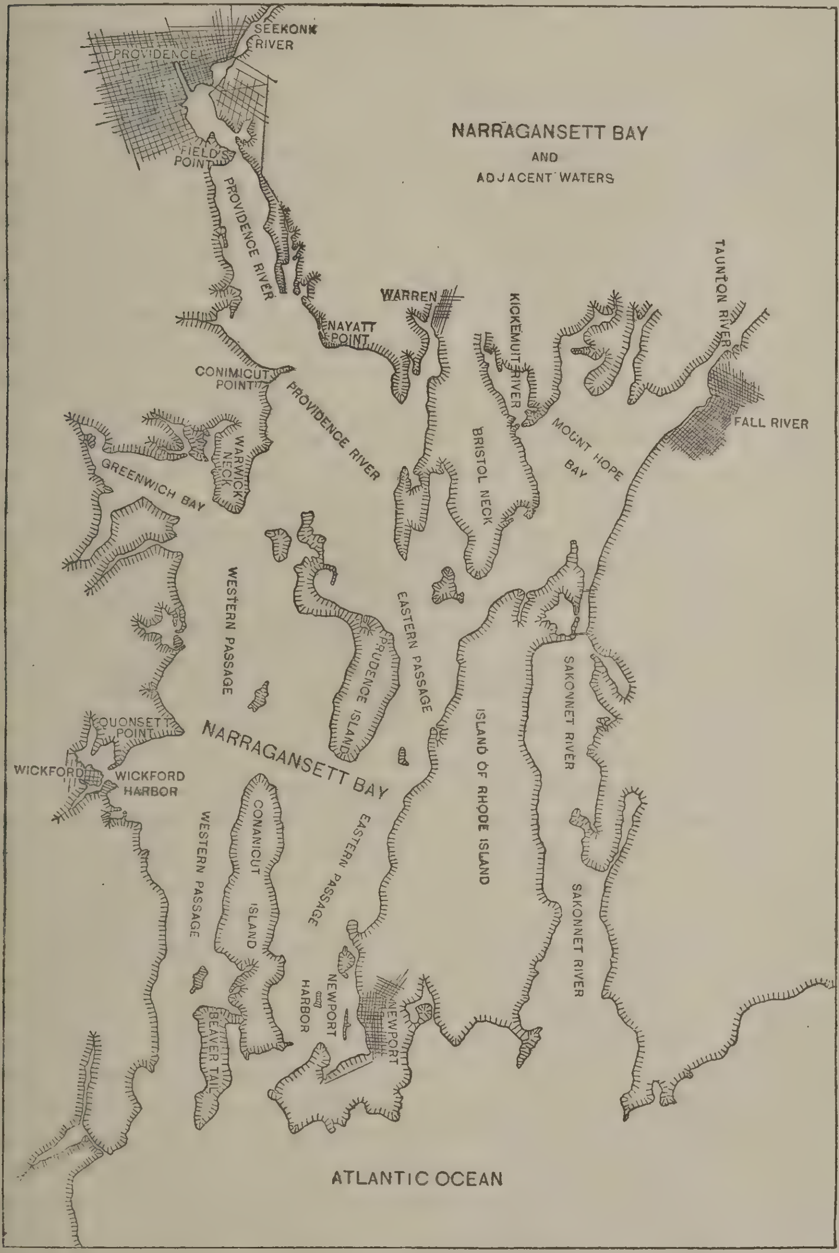
It was partially due to the interest stimulated by the splendid paper on "Oysters and Disease," by Prof. W. A. Herdman and Rupert Boyce, of Liverpool, England, that the present investigations were undertaken. At that time the city of Providence was discharging, daily, large quantities of "untreated" sewage into the Providence River. It is in this body of water that most of the so-called "Providence River oysters" are raised for market. Besides the sewage of Providence, the drains of numerous summer residences and shore resorts located on the river banks also contribute to the general pollution of these waters, and the sewage of the city of Fall River is a possible source of contamination to certain oyster beds situated in Mount Hope Bay.

Here, then, was an interesting and practically unexplored field for research, the careful study of which might throw some light on the question of sewage pollution of tidal waters and the contamination of shellfish growing therein. The experiments were begun in the fall of 1899, and continued through a period of about three years. The end in view was twofold: First, to obtain by bacteriological examination some definite knowledge of the extent of the pollution of these waters, and, second, to determine, also by bacteriological methods, the presence of sewage in oysters and other shellfish from various regions of the bay. *Bacillus coli* was taken as an indicator of sewage pollution, and samples containing this organism were considered to be contaminated by sewage.

The work was carried on at the Anatomical Laboratory of Brown University, under the direction of Prof. F. P. Gorham, to whom I desire to express especial gratitude for assistance and guidance throughout the entire investigation. I wish also to express my sincere thanks to Dr. A. D. Mead, of Brown University, and to Dr. H. C. Bumpus, director of the American Museum of Natural History, for material assistance and many kindnesses shown me during the preparation of this work.

DESCRIPTION OF NARRAGANSETT BAY.

The state of Rhode Island has an actual land area of 1,054.6 square miles. The waters of Narragansett Bay, with its tributaries, comprise an additional area of nearly 360 square miles, or more than one-fifth the total area of the state. Narragansett Bay proper is a narrow body of salt water that makes into Rhode Island from the Atlantic Ocean, which washes the southern border of the state, as will be seen by



reference to the accompanying outline map of the inland waters of Rhode Island, and gives the locations of its principal seaport cities and towns. The bay has an irregular coast line, and reaches inland in a general northerly direction for a distance of 25 miles. Its greatest width is about 7 miles. Its western boundary is formed by the mainland of the state; its eastern shores by the mainland and the island of Rhode Island, which separates the bay from the Sakonnet River. The upper part of the bay is considerably narrower than the lower, or southern portion, and for a distance of about 10 miles is known as the Providence River. At the head of this river is the city of Providence, which, with the surrounding towns, has a population of some 200,000. The Providence River at this point is joined by the Seekonk, a brackish stream which rises in Massachusetts. From 3 to 4 miles above its union with the Providence River the Seekonk flows through the city of Pawtucket, a city of nearly 40,000 inhabitants. For a distance of 6 or 7 miles below Providence the Providence River barely exceeds a mile in width, and in some places is much less than a mile wide. After passing Conimicut Point, a narrow tongue of land which juts abruptly out from the western shore, the river rapidly broadens to nearly three times its former width. Near this point the larger river receives the Warren River, a little stream less than half a mile wide, interesting in the present connection in that it is used for oyster culture. The towns of Warren, having a population of 5,100, and Barrington, 1,135, are situated on the banks of this stream, about 2 miles from its union with the Providence River.

Conanicut and Prudence islands, lying near the mid line of the bay proper, divide it into two strips of water called the East and West passages, respectively. The two entrances into the bay from the ocean are separated from one another by Beaver Tail, the southern portion of Conanicut Island, which juts out into the mouth of the bay between the mainland and the southwestern extremity of the island of Rhode Island. Of these two approaches to the bay, the one leads directly into the Western Passage, the other into Newport Harbor and thence into the Eastern Passage. Proceeding northward, the West Passage broadens very gradually till it reaches Quonset Point. Under the lea of this land lies Wickford Harbor and the town of Wickford. There are small oyster beds planted in the sheltered water of Wickford Harbor. Six miles above Quonset Point the Western Passage breaks up into two channels, one leading to the northwest into Greenwich Bay, the other in a northeasterly direction into the Providence River.

The city of Newport is situated on the island of Rhode Island, near the entrance to the East Passage to Narragansett Bay. This city, of 22,034 inhabitants, is one of the two large ports in Rhode Island waters, but is not of moment in the present connection, since it is situated at a considerable distance from the oyster beds of the bay.

There is an open waterway from the East to the West passages, between Conanicut and Prudence islands. Between Prudence Island and the island of Rhode Island the East Passage has somewhat the shape of a long funnel, with the broad, open end directed up river. Bristol Neck reaches down into this wide opening, dividing the passage into two channels, one to the northwest becoming continuous with the Providence River, and one to the northeast leading into Mount Hope Bay, an irregularly shaped expanse of water, about 7 miles long and a little over 4 miles in its greatest width, which receives the Kickemuit River at its northwest corner and the Taunton River from the northeast. As has been already stated, it joins Narragansett Bay through the narrow passage between Bristol Neck and Bristol Ferry, and the Sakonnet River through a still narrower cut between Common Fence Point and the mainland. The city of Fall River, having a population of nearly 105,000, is situated on the southern shore of the Taunton River, near its junction with Mount Hope Bay. Though a city of Massachusetts, Fall River is of interest in this connection because it discharges its sewage into the Taunton River, so that it is possible that pollution from this source might reach the oyster beds in more or less distant parts of the bay.

The water of the lower or southern part of Narragansett Bay varies from 50 to 150 feet in depth. The shores are for the most part rocky, and drop abruptly from the water line to a considerable depth, forming no areas that could be of value in the cultivation of oysters. But a very different formation is found in the upper portion of the bay. The water is shallow, not over 30 feet in mid-channel, and the shores are low and reach out to the channel with a very long and gentle slope. As might be expected, here are many sand beaches and numerous shoals, with 6 to 18 feet of water upon them, making excellent grounds for clams, oysters, mussels, scallops, and other shellfish, which are found in abundance. It is estimated that there are some 6,000 acres of this ground in the upper bay suitable for the cultivation of oysters.

Being in direct communication with the sea, the waters of Narragansett Bay are kept in constant circulation by tidal currents, which reach inland beyond Providence to the north and Fall River to the eastward. There is a rise and fall, mean average tide, of 4 feet and 6 inches at the wharves of Providence, Fall River, and Newport. In some portions of the bay especially strong currents are caused by the formation of the land in the immediate neighborhood. Such currents may be found in the narrow entrance to Newport Harbor, in the entrance to Mount Hope Bay, and in the "cut" leading from Mount Hope Bay into the Sakonnet River, where very large volumes of water have to pass through narrow openings. Lesser currents, due to a like cause, are found in the Providence River between Conimicut and Nayatt points, at the head of the Western Passage of the bay between

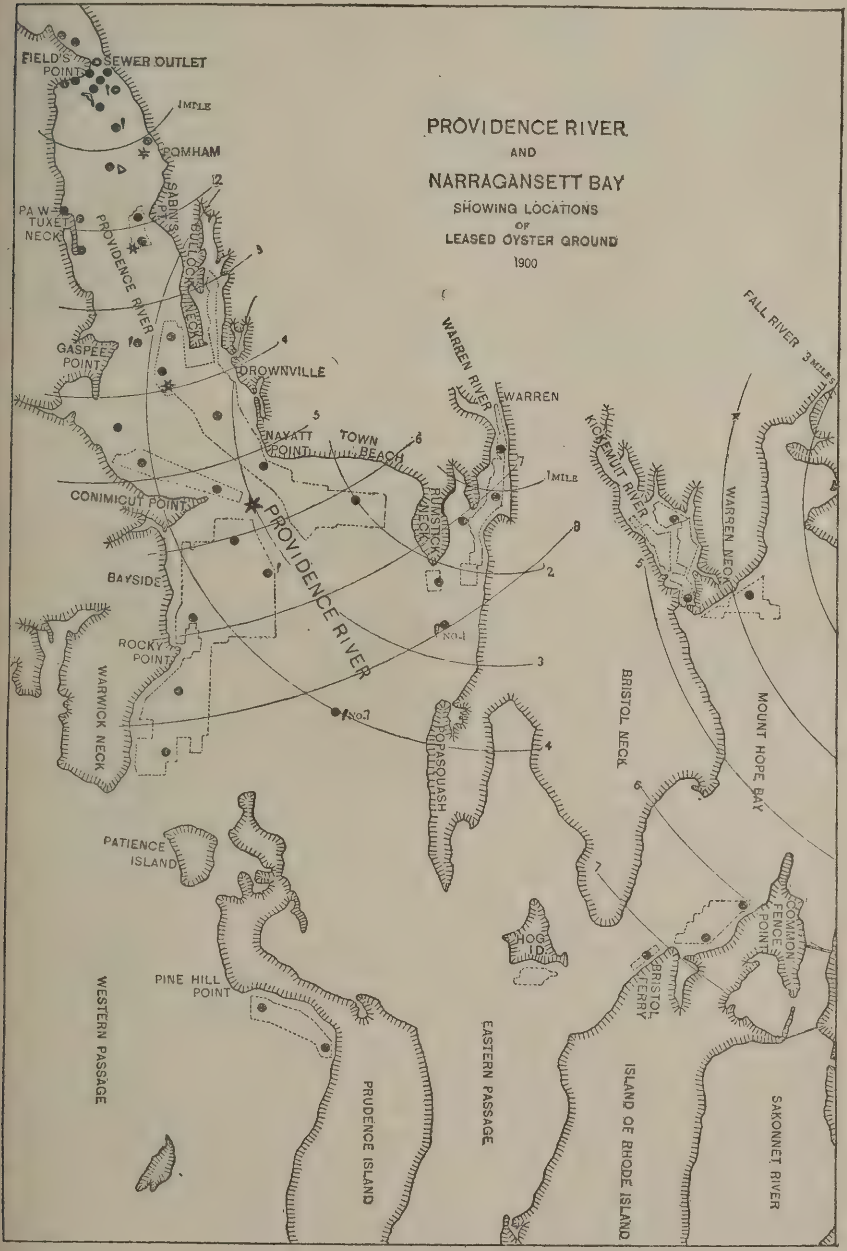
Warwick Neck and Prudence Island, and in several other localities. A description of the bay would hardly be complete without mention of these tidal currents, since a number sweep directly over some of the oyster beds and carry with them whatever pollution may have entered the water.

THE LOCATION OF THE LEASED OYSTER GROUND IN NARRAGANSETT BAY.

In the first annual report of the Commissioners of Shell Fisheries of Rhode Island it is stated that the income from the oyster ground leased in the Providence River during the year 1864 amounted to $61. In 1900 more than 3,000 acres of land in the Providence River and Narragansett Bay were devoted to this branch of industry, yielding an income of $25,000. The figures for the year 1903 show an increase of 2,000 acres in the total area leased in that year, with a rental increased to nearly $45,000.

The map on page 203 indicates the location of the leased oyster ground for 1900–1901, since the larger part of the oysters examined in this investigation were collected in the latter year. The areas occupied by the beds are outlined in dotted lines. It will be observed that the most extensive grounds are found in the Providence River rather than in the deeper waters of Narragansett Bay—in fact, there were but two oyster beds of any importance at this time in the bay proper. One was located in Wickford Harbor, 21 miles south of Providence; the second, under the western shore of Prudence Island, about 14 miles from the capital city. The Wickford layings, not represented on the map, comprise some five or six acres of land planted in 6 to 10 feet of water, in the direct path of a constantly flowing tidal current. No sewage is discharged into this body of water, so that the beds are free from local contamination. As will be seen by reference to the map, the Prudence Island beds are located south of Pine Hill Point, in a bend of the land formed by the irregular coast line of the island. They occupy an area of some 300 acres. There is a hard sand or gravel bottom in this locality, which is covered by from 10 to 15 feet of water at mean low tide. These beds are far removed from pollution of any sort, the nearest human habitation being at least 3 miles distant from this region.

As has already been stated, the most extensive oyster grounds are located in the Providence River. These are two large areas, over 1,000 acres each in extent, which lie between Warwick Neck and Bullock Neck. The first of these areas, known as the Rocky Point oyster beds, lies on the western side of the ship channel, and extends from near the southern shore of Warwick Neck northward to the southern boundary of Conimicut Point, the northern limit of the beds approaching to within 7¾ miles of Providence. These layings occupy a shoal that makes out from the western shore of the river, and is covered by water varying in depth from 6 to 20 feet at low tide. Below Conimicut Point the river is fully 3 miles in width, and has free communication



with both the Eastern and Western passages of the bay, insuring a good circulation of water at all times; but above this point it is scarcely a mile wide, and is an open body of water, unobstructed by islands or other land formation until it reaches Fields Point, 1¾ miles below Providence, where the sewage of the city is discharged into the river. Thus the second of these large areas lying on the eastern side of the channel reaches well up into this narrower section of the river. The southern layings of this area, the Nayatt oyster beds, are located south of Town Beach, well over toward Rumstick Neck, in the broader portion of the river, and from this region extend in a westerly direction around Nayatt Point, then, following the eastern shore, in a northerly direction to within 5¼ miles of the city. The northern section of the area is spoken of as the Bullock Neck oyster beds. While the southern sections lie in the more open water of the lower river, the northern limits are well up in the more confined waters of the upper river, therefore in the direct path of any contamination that may be borne downstream by the tides. (See map.)

Still another bed, of some 300 acres' extent, is located on the western side of the river, directly north of Conimicut Point, occupying a long narrow strip of land that extends from near the low tide limit to Conimicut Light, which marks the western boundary of the ship channel. It is about 7 miles distant from the city of Providence. The list of oyster grounds in the Providence River is completed with the description of a bed of about 125 acres, located on the eastern side of the river, directly off Sabins Point. As may be seen by reference to the map, this bed is but 3 miles below the city limits.

Before leaving the neighborhood of the river, however, the beds in the Warren River must be mentioned. In 1900 extensive layings of oysters were made in and about the entrance of this stream. Near the mouth, they were confined to the shoal water on either side of the channel, but a short distance above this section they occupied both channel and shoal water, so that the bottom of the river was a continuous oyster bed from near its junction with the Providence River to the town of Warren, 2 miles inland.

The five remaining oyster beds located in Rhode Island waters are found in or near Mount Hope Bay. The first of these is planted on a shoal directly south of Hog Island, which is less than a mile from the entrance of the bay; the second is a much smaller area off Bristol Ferry, in the narrow entrance to the bay; the third and largest area lies to the east of Bristol Ferry, off Common Fence Point; the fourth, 3 miles distant from the last-mentioned bed, in the northwestern corner of the bay, south of Warren Neck; and the fifth, around Warren Neck, in the Kickemuit River. The beds off Warren Neck are 4 miles from the city of Fall River; those at the entrance of the bay, 7 miles. Thus it may be noted that these grounds are situated at a considerable distance from the discharge of the Fall River sewer. The beds in the entrance of the bay are also scoured by very strong tidal currents, due to causes already explained.

THE SOURCES OF SEWAGE POLLUTION OF NARRAGANSETT BAY.

The sewage of the city of Providence and immediate neighborhood is collected at the sewage pumping station at Fields Point, about 1¾ miles below the city, and is discharged into the river through a single large main. The outlet of this drain is indicated on the map on page 203. Though it is covered by 25 feet of water at low tide, it may be readily located on a calm day by the greasy, turbid stream of sewage which rises rapidly to the surface of the water at that point.

In 1900 an average amount of nearly 14,000,000 gallons of sewage was daily pumped into the river through the Fields Point sewer, and at that time was thrown into the river in a "crude" or "untreated" state; that is, it was simply passed through a screen to remove the solid matters before being sent on to the outfall pipe. The screen consisted of a rack or frame of parallel iron rods placed about an inch apart, and was set at an angle of about 20 degrees from the perpendicular. The stream of sewage was allowed to flow through this apparatus, and whatever constituents were too bulky to pass through the grating were scraped off with a rake and otherwise disposed of; but, as might be expected, considerable amounts of solid matter slipped through, and the beaches within half a mile of the outlet were strewn with this refuse.

Before these investigations were completed, however, the city put in operation a sewage disposal plant at the Fields Point Station. After screening, as already described, the sewage is subjected to the action of sulphate of iron and chloride of lime, which process causes about 50 per cent of the total solids then in suspension to precipitate out and sink to the bottoms of large reservoirs in which the sewage is allowed to remain for twenty-four hours. The clearer fluid is then drawn off and turned into the river, while the "sludge" which remains on the bottom of the basin is strewn over the land. Before this method was employed the gray scum from this sewer could often be traced on the surface of the water several miles below Fields Point, where it had been carried by tidal currents. The station has one other large outlet, the "storm" sewer, which opens into the river from the northern shore of the point. This sewer is a culvert built in the form of an arch, which is entirely out of water at low tide. It is used only in case of accident to the large main or during heavy rain storms, when 20,000,000 and sometimes 25,000,000 gallons are discharged in twenty-four hours.

Two other small overflow sewers help to drain the east side of the city, pipes discharging into the Seekonk River between Red Bridge and Washington Bridge. The sewage from these drains, together with that of the city of Pawtucket, situated on this same stream 3 miles above Providence, and the waste from the various mills along the banks of the river pass down the Seekonk into the head of the Providence

River. Also, since Providence and Pawtucket are manufacturing centers, a large amount of waste from gold and silver refineries, from bleacheries and dye houses, and coal tar products from the gas companies' plants, ultimately find their way into the river.

In addition to these more important sources of pollution, the drains from numerous shore resorts and summer residences situated on the river banks must be named as a secondary source of contamination. These drains are of minor importance in the general contamination of the water, since the amount of sewage discharged by them is small in comparison with that already mentioned, and also because they are in use but a few months during each season, and at a time when few oysters are dredged for market.

The section of the river which receives this large amount of sewage is a strip of water a little over 5 miles long, varying from about 1 mile to 1¼ miles in width. As has already been pointed out, the tide reaches well up into the river past Providence and up the Seekonk River nearly to Pawtucket. Thus twice in every twenty-four hours clean sea water from the bay below flows toward the polluted areas, and is a very important factor in the purification of the river.

Much more space has been devoted to the description of the conditions in the Providence River than will be given to the other waters of the bay, because this river is more polluted by sewage, and because most of the oyster ground of Rhode Island waters is located in this body of water. The pollution of the Warren River is of only local importance, since it is soon swallowed up in the large volume of fresh sea water it encounters when this stream joins the Providence River. The contamination of the Warren River is due chiefly to mill waste and to the sewage from a few private drains that discharge into the river.

The sewage of Fall River is the third factor in the pollution of the bay. This waste is discharged into the Taunton River near the head of Mount Hope Bay. The outfall of this sewer is, of course, at a considerable distance from the Providence River and Narragansett Bay, and even though a large quantity of sewage and mill waste is passed into the Taunton River, all visible evidence of pollution has disappeared from the water at the entrance of Mount Hope Bay, nearly 7 miles distant from the sewer outfalls.

These three sources, then—the Providence sewers, the Warren mill waste, and the Fall River sewers—are the principal ones from which contamination can be spread to the oyster beds of the river and bay. The sewage from Newport never reaches the oyster beds, the nearest of which are at least 12 miles above Newport Harbor.

BACTERIOLOGICAL ANALYSIS OF WATER SAMPLES FROM NARRAGANSETT BAY.

Methods employed.—The usual methods for isolation of *B. coli* from water and sewage were used in this work. Fermentation tubes containing a neutral 1 per cent dextrose broth were inoculated with 1 c. c. of the suspected water and incubated for three days at 37° C. In some cases a 0.1 per cent phenol broth was also inoculated with 1 c. c. of the water and allowed to develop at 37° C. for twenty-four hours. In a few tests litmus-lactose-agar and agar containing 1 per cent neutral red were sown with varying amounts of water and grown at incubator temperature.

If no gas was formed in the fermentation tubes in twenty-four hours the test was considered negative without further procedure. If, however, any considerable quantity of gas developed within this time, litmus-lactose-agar plates were inoculated in most cases from these tubes and incubated at 37° C. twenty-four hours longer. When litmus-agar plates were not used, a gelatin medium was substituted. Any red colonies developing on the litmus medium, and any colonies showing the characteristic growth of *B. coli* on gelatin, were fished out and transferred to slant agar tubes. From the cultures thus obtained subcultures were made in neutral dextrose and lactose broth, nitrate solution, milk, sugar-free broth containing 2 per cent of peptone and gelatin. When growth occurred in the phenol broth, although sufficient gas to indicate the presence of *B. coli* was not developed in the fermentation tubes, litmus-lactose-agar plates were inoculated from the phenol broth and treated as already described.

Organisms giving the following positive reactions to tests were regarded as members of the colon group of bacteria:

1. A small more or less motile bacillus in twenty-four-hour bouillon or agar cultures. Usually not all the bacilli in one microscopic field are motile—often sluggishly motile.

2. Fermenting dextrose broth with the production of gas. The large part, if not all, of the gas is formed during the first twenty-four hours. The liquid in the tube must be distinctly acid to indicate *B. coli*. The ratio of hydrogen to carbon dioxide is approximately 2 to 1. This ratio is, however, more or less variable in cultures from a single strain. The total amount of gas produced in dextrose usually does not exceed 55 per cent, though there is also more or less variation in this characteristic.

3. Fermenting lactose with the production of much gas; reaction strongly acid.

4. Indol produced in sugar-free broth containing 2 per cent of peptone.

5. Milk coagulated in three days at room temperature; in twenty-four hours at 37.5° C.; casein not liquefied; reaction acid.

6. Gelatin not liquefied; stab cultures and plate cultures give characteristic growths.

7. Nitrates reduced to nitrites.

Bacterium lactis aerogenes is a closely allied form, but differs from *B. coli* in that it is nonmotile; it produces larger amounts of gas in dextrose broth (75 per cent), and it does not produce indol. It is nonpathogenic.

B. cloacæ also produces large quantities of gas in dextrose bouillon (from 65 to 75 per cent). It liquefies gelatin, casein, and blood serum, and produces indol and nitrates.

Samples of water to be tested were collected in sterile 25 c. c. tubes by means of an apparatus similar to that suggested by Professor Bolley for use in deep wells. The tubes were made from large 8-inch test tubes by drawing out slightly in a Bunsen flame the open end of the tube, bending the lengthened portion to a right angle with the rest, and finally drawing it out into a fine capillary tube. These tubes were sterilized, and after a partial vacuum had been secured by heating, the fine tube was sealed in a flame. A rack holding 20 of these tubes was easily carried in a small grip. The collecting apparatus consisted of a solid block of brass 9 inches long by 1½ inches wide by three-fourths inch thick, against the flat side of which the tube was firmly held by two sets of clamps, the sealed capillary tube passing through a hole bored in the upper end of the block. In collecting the water samples the apparatus was lowered by a stout cord to the desired depth and the sealed tube broken by a metal slide, which was operated by allowing a weight to run down the line on which the apparatus was lowered. The partial vacuum in the tubes usually filled them one-half to three-fourths full of water. These tubes were again placed in the rack and carried to the laboratory unsealed, for a length of the bent tube sufficient to protect the sample from outside contamination usually remained after the sample had been collected. When the tubes reached the laboratory, at no more than four or five hours after collection of the water samples, the tops were passed through a flame and enough of the glass broken away with sterile forceps to allow the entrance into the tube of a sterile 1 c. c. pipette. Samples were immediately transferred from these tubes to the different culture media, as already described.

When samples were taken in deep water, two collections were usually made at each locality visited, one a foot below the surface of the water and a second a foot off the bottom of the river. In the shallow water near the shores samples were collected by plunging sterile bottles below the surface of the water. In examining clam flats and mussel beds left uncovered by the tide, samples of sand and mud were collected at low tide and samples of the water covering these grounds on the flood tide.

Results.—The bacteriological examination of any large body of water resolves itself into an analysis of series of samples taken from various sections of the stream. So in this survey of the Providence River collections were made first at the head of the river, then, proceeding downstream, at intervals of varying distance until the polluted area was passed. For the sake of brevity, the localities at which collections were made will be spoken of hereafter as "stations," and they have been indicated on the map by large dots. When possible, they were chosen near some prominent landmark, so that they might be more easily found a second time, since in many instances several trips were made in order to observe the effect of varying conditions of tide and weather.

The evidences of sewage pollution of both the water and shore in the neighborhood of Fields Point were very obvious. Below the point the west bank of the river falls abruptly away from the eastern shore, taking a westerly direction for nearly half a mile, when it again resumes its general southerly course. The deep water of the river follows closely the eastern shore, so that a large area of shoal water is formed south of Fields Point, extending out beyond Starve Goat Island. This section of the river is therefore out of the direct tide current, which naturally follows the channel along the east shore, and the water is consequently rather sluggish. It is a dirty gray color, which is due to the large amounts of sediment in suspension. Also the beaches within a quarter of a mile of the sewer outfall are usually covered with foul-smelling slime and collections of sewage refuse, left there by the receding tide water. Before the Fields Point sewage station was put into operation this shoal was a famous natural oyster bed, but it has been abandoned for a number of years. At the present time quantities of seed oysters are taken from this locality in the spring of the year and planted on beds farther down the river. The beaches in this neighborhood also produced an abundance of clams.

In the section of the river above described the first series of water samples was collected at 11 stations situated in an area at no point more than half a mile distant from the outlet of the main sewer. Commencing on the northern shore of Fields Point, these stations were located as follows: Station 1, halfway between the ship channel and the northern shore of the point, directly off the outfall of the "storm" sewer; station 2, off the end of the steamboat landing; station 3, directly over the outlet of the main sewer; stations 4 and 5, in deep water on opposite sides of the channel, a short distance below the point; stations 6, 7, and 8, in the shallow water on the flats running out from the southern shore of the point; stations 9 and 10, in about 10 feet of water near Starve Goat Island, and station 11, in the ship channel just off buoy No. 11. Perhaps the exact situations can be better understood by reference to the map. Two trips were made to these localities during the winter and spring of 1901, and three more stations were located about this time in the river near Pomham—one west of the ship channel, close by buoy No. 9; another on the eastern side of the channel, directly north of Pomham Light, and a third in the more shoal water to the west of Pomham Beacon, which is about 1¼ miles below Fields Point. Three trips were made to the stations. The results of the analyses of the samples are arranged in the accompanying tables.

TABLE I.—*Analysis of water samples collected in the neighborhood of Fields Point.*

Date and station.	Dextrose, fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
January 15, 1901, tide rising three-fourths high; wind fresh SW.				
Station 1, surface tube	+	+	+	+
deep tube	+	+	+	....
deep tube	+	....	....	....
2, surface tube	+	(a)	+	....
deep tube	+	+	+	+
3, surface tube	+	+	+	+
deep tube	+	+	+	....
4, surface tube	+	+	+	+
deep tube	+	+	+	....
5, surface tube	+	+	+	+
deep tube	+	+	+	+
6	+	(a)	+	+
7	+	+	+	+
8	+	+	+	....
January 29, tide rising; one-half mile from sewer.				
Station 9, surface tube	+	+	+	+
deep tube	+	+	+	....
10, surface tube	+	+	+	....
deep tube	+	+	+	....
11, surface tube	+	+	+	....
deep tube	+	+	+	....
April 10, low tide.				
Station 1, surface tube	+	+	+	+
deep tube	+	−	−	+
2, surface tube	+	+	+	....
deep tube	+	+	+	....
3, surface tube	+	+	+	....
4, surface tube	+	+	+	....
deep tube	−	−	−	....
5, surface tube	+	+	+	....
deep tube	+	(a)	+	....
6	+	+	+	....
7	+	+	+	+
8	+	+	+	+
April 24, low tide.				
Station 9, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
10, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
11, surface tube	+	+	+	....
deep tube	+	−	−	....

[a] Not made.

TABLE II.—*Analysis of water samples collected off Pomham.*

Date and station.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
February 26, flood tide.				
Station 1, surface tube	+	+	+	+
deep tube	+	+	+	+
2, surface tube	+	+	−	+
deep tube	+	+	+	....
3, surface tube	+	+	+	....
deep tube	+	+	+	....
March 2, tide falling.				
Station 1, surface tube	+	+	+	....
deep tube	−	−	−	....
2, surface tube	+	(a)	+	....
deep tube	+	+	+	....
3, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
March —, tide falling.				
Station 1, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
2, surface tube	+	+	+	....
deep tube	+	−	−	+
3, surface tube	+	+	+	+
deep tube	+	+	+	+

[a] Not made.

The data given in Tables I and II show clearly that the water of the river in the immediate neighborhood of Fields Point and also at Pomham, 1¼ miles below this point, is polluted by sewage to a very considerable extent, since it is possible to isolate *B. coli* from practically every sample collected within this area. This statement seems to be trustworthy whether the tide is making upstream or falling, or whether the samples are collected when there is a stiff breeze from the southerly direction, thus tending to drive an increased amount of water up the river, or during a flat calm.

An attempt to estimate the number of bacteria per cubic centimeter in the water about Fields Point was made by inoculating nutrient gelatin plates from two of the samples collected April 10 and keeping them three days at room temperature. Four plates were made from each sample, and the average count of colonies developing was estimated as follows: Station 2, surface water, 1,500,000 bacteria per cubic centimeter; station 3, surface water, 2,000,000 bacteria per cubic centimeter. Thus the quantitative as well as qualitative analysis points to high organic pollution of these waters.

The second series of samples was collected from a section of the river from 2 to 2½ miles from Fields Point. These samples were taken at five stations, as follows: Station 1 over the northern part and station 2 at the southern end of the oyster grounds off Sabins Point; on the Pawtuxet shore, station 3 in the shallow water covering the sand beach west of the Rhode Island Yacht Club house, station 4 off the end of the club wharf, and station 5 in shallow water again, from the shore near the end of Pawtuxet Neck. Four trips were made to the first two of these stations; but one to those on the western shore of the river. The result of these analyses is included in Table III.

Table III.—*Analysis of water samples collected off Pawtuxet Neck and over the Sabins Point oyster beds.*

Date and station.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
March 18, tide falling.				
Station 1, surface tube	+	+	+	....
deep tube	+	+	+	....
2, surface tube	+	+	+	....
deep tube	+	?	?	....
March 19, tide falling.				
Station 1, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
2, deep tube	+	(a)	+	....
April 10, tide falling.				
Station 1, surface tube	+	(a)	+	....
deep tube	+	(a)	?	....
2, deep tube	+	....	—	....
April 17, tide rising, near flood.				
Station 1, surface tube	+	(a)	+	+
deep tube	—	+	—	....
2, surface tube	+	+	—	....
deep tube	—	(a)	—	....
March 27, low tide.				
Station 3	+	+	+	+
4	+	+	+	....
5	+	+	+	+

[a] Not made.

In this section of the river the water is apparently much cleaner along the eastern shore over the oyster bed, while the condition on the Pawtuxet shore was evidently so bad that only one collection of samples was made at this point. Still, analysis of samples from stations 1 and 2 showed that *B. coli* was usually present in the water through this section of the river, at least during a falling tide, though only one of the four samples taken April 17 was found to be contaminated. It must be remembered, however, that these samples were taken at flood—the time, if ever, that the river will be free from pollution.

A mile and a half below the Sabins Point ground are found the northern limits of the extensive Bullock Neck beds. No samples were collected in that portion of the river between these beds, but five stations were located below this point over the oyster bed that extends along the eastern shore, past Drownville and Nayatt Point to Town Beach, and four on the western side of the river. These stations were located as follows: Station 1, at the northern end of the Bullock Neck oyster bed, 3½ miles below Fields Point; station 2, to the north of Bullock Point Light; station 3, off the Drownville shore, 4½ miles from the sewer outlet; station 4, directly south of Nayatt Point; and station 5, on the southern portion of these oyster layings, well over toward Rumstick Point. On the western side of the river, station 6 was placed at buoy No. 3; station 7, at buoy No. 1; and stations 8 and 9, over the oyster beds off the north shore of Conimicut Point. Several trips were made to this section in the spring and fall of 1901. The results obtained from the analysis of the samples may be tabulated as follows:

TABLE IV.—*Analysis of water samples collected on the Bullock Neck, Nayatt Point, and Conimicut Point oyster beds.*

Date and station.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
March 30, tide rising.				
Station 1, surface tube	+	+	+	....
deep tube	−	−	−	....
2, surface tube	+	+	−	....
deep tube	+	+	+	....
3, surface tube	+	+	−	....
deep tube	+	+	−	....
6, surface tube	+	(a)	+	....
deep tube	+	+	−	+
7, surface tube	−	+	−	....
deep tube	+	+	+	....
May 2, tide falling.				
Station 1, surface tube	+	+	+	....
deep tube	−	+	−	....
2, surface tube	+	(a)	+	....
deep tube	+	+	+	....
3, surface tube	+	+	−	....
deep tube	+	+	+	....
May 15, tide falling.				
Station 1, surface tube	+	+	+	....
deep tube	+	+	+	....
2, surface tube	+	+	+	....
deep tube	−	+	−	....
3, surface tube	+	+	+	....
deep tube	+	+	+	....
4, surface tube	+	−	−	....
deep tube	+	+	−	....
5, surface tube	−	+	−	....
deep tube	+	+	+	....
October 11, tide rising.				
Station 8, surface tube	+	+	−	....
deep tube	+	+	+	....
9, surface tube	+	+	−	....
deep tube	−	−	−	....
October 24, tide rising.				
Station 4, surface tube	+	+	+	....
deep tube	−	+	−	....
5, surface tube	+	+	−	....
deep tube	+	+	−	....
8, surface tube	+	(a)	+	....
deep tube	−	+	−	....
9, surface tube	−	+	−	....
deep tube	−	−	−	....
October 29, tide falling.				
Station 4, surface tube	+	−	−	....
deep tube	+	(a)	+	....
5, surface tube	+	+	−	....
deep tube	−	−	−	....
8, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
9, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
November 3, tide rising.				
Station 4, surface tube	+	+	+	....
deep tube	−	+	−	....
5, surface tube	+	+	+	....
deep tube	−	−	−	....
8, surface tube	+	+	+	....
deep tube	+	+	−	....
9, surface tube	+	(a)	+	....
deep tube	−	+	−	....

[a] Not made.

Though the river below Bullock Neck does not have the appearance of a sewage-polluted stream, it is possible to isolate *B. coli* from some samples of water taken from it. A smaller percentage of the samples collected about Conimicut and Nayatt points than of those collected nearer the sewer outlet give tests for this organism; it was found in 59 per cent of the water samples taken in the neighborhood of Bullock Neck; in 50 per cent of those collected over the Conimicut beds; and in but 31 per cent of those obtained from the Nayatt ground. It is very evident that the tides play an important part in the purification of this section of the river. Most of the samples which gave positive reactions for *B. coli* were collected on a falling tide. Samples taken on the flood are, in many cases, free from sewage bacteria.

Here, then, is an area from 3 to 6 miles distant from the chief sources of pollution, in which the sewage, when present, is diluted to such an extent that examination often fails to reveal the presence of fecal bacteria in 1 c. c. samples.

Below Conimicut Point, in the broader expanse of the lower river, five stations were located over the extensive Rocky Point oyster ground. These were situated as follows: Station 1, over the northern portion of these grounds, 6 miles below Fields Point; station 2, about half a mile farther south, near channel buoy No. 9; station 3, just north of Rocky Point; station 4, south of Rocky Point; and station 5, over the southern areas of this ground, which is about 8½ miles south of the Fields Point sewer outlet.

A single trip was made to the Warren River in October, 1902, and samples collected at five stations about half a mile apart. Station 1 was located at buoy No. 1, marking the entrance to Warren River channel, which is in reality in the Providence River, about half a mile directly south of Rumstick Neck; station 2 directly in the entrance to the Warren River, half a mile above station 1, and so on up the river. At this time samples were collected at a station, No. 6, in Providence River, located at buoy No. 7. These samples were taken because this locality is swept by any tidal currents that may come from the Warren River on ebb tide, and it was desired to ascertain whether pollution from this stream was noticeable in the Providence River at that point. Tables V and VI give in condensed form the results of the analysis.

TABLE V.—*Analysis of water samples collected over the Rocky Point oyster ground.*

Date and station.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
October 10, 1901, tide rising.				
Station 1, surface tube	−	−	−	....
deep tube	+	−	−	....
2, surface tube	−	−	−	....
deep tube	−	+	−	....
3, surface tube	+	+	−	....
deep tube	−	−	−	....
4, surface tube	−	−	−	....
deep tube	−	−	−	....
5, surface tube	−	+	−	....
deep tube	−	−	−	....
October 19, tide falling (very low).				
Station 1, surface tube	+	+	+	....
deep tube	+	−	−	....
2, surface tube	+	+	−	....
deep tube	−	+	−	....
3, surface tube	−	−	+	....
deep tube	−	+	+	....
4, surface tube	−	+	−	....
deep tube	−	−	−	....
5 surface tube	+	−	−	....
deep tube	+	+	−	....
November —, 1901, tide falling.				
Station 1, deep tube	−	−	−	....
2, deep tube	+	+	−	....
3, deep tube	−	−	−	....
4, deep tube	−	−	−	....
5, deep tube	−	−	−	....

TABLE VI.—*Analysis of water samples collected in Warren River.*

Date and station.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
October 8, 1902, tide falling.				
Station 1, surface tube	+	(a)	+	....
deep tube	+	(a)	+	....
2, deep tube	+	(a)	+	....
3, deep tube	+	(a)	+	....
4, deep tube	+	(a)	+	....
5, surface tube	+	+	+	....
deep tube	−	−	−	....
6, surface tube	−	+	−	....
deep tube	−	−	−	....

[a] Not made.

From the above data it is apparent that the river after passing Conimicut Point is comparatively free from sewage. *B. coli* was found in only two samples taken from this section, once in a sample from station 1 and in one sample from station 3; both these samples were collected at a very low tide, due to the change of moon. On the other hand, it will be noticed that nearly all the samples from the Warren River gave tests of *B. coli*, but after the Warren River joins the Providence River this organism soon disappears from the water.

Thus between 6 and 7 miles below the Fields Point sewer is another area of water from which nearly all traces of pollution have disappeared. *B. coli* is found only occasionally, and then on the ebb tide. Another zone of pollution spreads out from Warren River, however, and at station 1, buoy No. 1, 3 miles below the town of Warren, evidences of it can be discovered; but at station 6, 4 miles below this town, examination fails to reveal the presence of sewage bacteria. Samples from the Prudence Island and Wickford oyster beds contained no colon bacilli; neither did the samples collected over the southern parts of the Rocky Point oyster ground, so that the Providence River, 8 miles below the chief sources of contamination, ceases to be a polluted stream. If sewage is present in the water below this point, it is in too great dilutions to be recognized in the 1 c. c. samples that were used in this work. The waters of Narragansett Bay are also free from sewage pollution.

The next series of samples was collected in or near the Kickemuit River. Three stations were located in this part of the bay: Station 1, over the oyster layings south of Warren Neck; station 2, just inside the mouth of the Kickemuit River; and station 3, about three-quarters of a mile farther up the river. *B. coli* was found in but one sample from the oyster beds south of Warren Neck, which are 4 miles from the Fall River sewer. Finally, three stations near the entrance to Mount Hope Bay were visited—stations 1 and 2, over the oyster bed north of Common Fence Point, 6 and 6¾ miles, respectively, from Fall River; and station 3, over the Bristol Ferry bed, 7¼ miles below the city. But a single sample contained the colon bacillus. This was collected at station 3, off Bristol Ferry, and the presence of the organism was probably due to some local contamination rather than to the sewage from Fall River, since samples from stations 1 and 2, considerably nearer the chief source of pollution, did not contain this bacillus.

As a final test of the distribution of sewage bacteria in the river, an attempt was made to estimate the number of colon bacilli per cubic centimeter in this water. For this test lactose agar plates containing 1 per cent neutral red were inoculated directly with the water to be tested, and incubated forty-eight hours at 42° C. After this period, the colonies developing were examined, and those exhibiting the characteristic appearance of *B. coli* on this medium were counted. It was impossible to study each separate colony in pure culture, therefore these figures have but an approximate value. Four plates were made from each sample; and the figures given in Table VII represent the average number of characteristic colonies developing within the given time. The samples used in this test were collected (October 21, 1901, tide rising) at the stations indicated on the map on page 203. The first sample was taken near the sewer outfall at Fields Point; the others at places farther down the river.

TABLE VII.—*Quantitative analysis of water samples collected in Providence River.*

Locality.	B. coli per cubic centimeter in river water.
Fields Point:	
Station 3, surface tube	96
deep tube	(?)
9, surface tube	450
deep tube	500
10, surface tube	200
deep tube	50
Pomham:	
Station 1, surface tube	75
deep tube	14
3, surface tube	9
deep tube	14
Bullock Neck:	
Station 2, surface tube	24
deep tube	0
6, surface tube	20
deep tube	6
Bullock Neck:	
Station 7, surface tube	2
deep tube	6
4, surface tube	3
deep tube	2
5, surface tube	0
deep tube	0
8, surface tube	1
deep tube	0
Rocky Point:	
Station 1, surface tube	0
deep tube	0
2, surface tube	0
deep tube	0
3, surface tube	0
deep tube	0

Though the results obtained by this method are by no means as accurate as might be desired, nevertheless they indicate, as has already been proved, a gradual decrease in the amount of pollution in the river as it reaches down toward the headwaters of Narragansett Bay.

The data obtained by the analysis of the foregoing water samples may be summed up as follows: The Providence River above Conimicut Point is a sewage-polluted body of water, but below this point the water of the river and the headwaters of Narragansett Bay are free from contamination. The presence of sewage may also be detected in Warren River. That section of Mount Hope Bay in which the oyster ground is situated appears to be entirely free from pollution.

The distribution of sewage in Rhode Island waters, as indicated by the presence of *B. coli* in water samples, may be readily traced from the principal sources of contamination. As has already been stated, these are three in number—the Providence sewage, Warren mill waste, and the sewage from Fall River. Referring once more to the map on page 203, it will be observed that equidistant concentric lines radiating from three different centers have been drawn across the areas representing the Providence River, the Warren River, and Mount Hope Bay. The space between these lines represents 1 mile actual distance. The series of arcs which divide the Providence River into sectors are drawn using the point at which the outlet of the Fields Point sewer is represented as a center, and with their aid the distribution of sewage in the river may be traced as follows:

The water of the river in the sector included within the arc of the first circle, at no point more than 1 mile distant from Fields Point, is highly polluted. Samples taken from this portion of the river contain *B. coli* and other fecal bacteria under all conditions of tide and weather.

These organisms are also found in the majority of samples collected 2 miles below the sewer outlet. About 74 per cent of these samples contained *B. coli.*

The section of the river 2 to 4 miles below the chief source of contamination is much freer from pollution. *B. coli* was found in 59 per cent of the samples from this area, and the majority of positive tests was obtained from samples collected at low water, while many samples taken on the flood tide did not contain fecal bacteria of any sort.

Five miles below Fields Point, still fewer samples contain *B. coli;* 6 miles below this organism is rarely found. About 50 per cent of the samples from the Conimicut Point oyster beds and about 31 per cent of those from the Nayatt Point ground contained colon bacilli. The eastern shore of the river is cleaner than the western above Conimicut Point. Below this point reverse conditions are encountered. No fecal bacteria were found in samples collected on the Rocky Point oyster ground, over 7 miles distant from Fields Point.

But *B. coli* was isolated from samples taken near buoy No. 1, marking the entrance of the Warren River channel. This station is a little over 2½ miles distant from the town of Warren, and at the same time is far enough out in the Providence River to be just included in the arc which marks the 8-mile limit from Fields Point. No fecal bacteria were found in samples collected near buoy No. 7, however, which is 4 miles distant from Warren and about 8½ from the Providence sewer outlet. Thus it may be stated that 8 miles below the Fields Point sewer no colon bacilli have been found in the water of the river, and from this point on till it reaches the headwaters of Narragansett Bay the river is free from sewage bacteria.

Passing downstream, the number of colon bacilli in the river water decreases gradually from 500 per cubic centimeter one-half mile below Fields Point to 1 per cubic centimeter at Conimicut. Below Conimicut no sewage bacteria were found by the plate method employed in this test.

The water on the Prudence Island and Wickford oyster beds does not contain *B. coli.*

The portion of Mount Hope Bay included in the Rhode Island territory is comparatively free from pollution, and the oyster ground in this bay is located from 4 to 7 miles below Fall River, the chief source of pollution. *B. coli* was isolated from a single sample taken in the entrance to the bay, but the presence of the organism in this instance was probably due to local contamination, for other samples taken near by did not give reactions for colon bacteria.

Samples from Kickemuit River did not contain *B. coli.*

One sample collected on the oyster bed under Warren Neck contained *B. coli.*

BACTERIOLOGICAL ANALYSIS OF SHELLFISH FROM NARRAGANSETT BAY.

Methods employed.—Much the same plan of work was employed in the examination of the shellfish of Narragansett Bay as was used in the water analysis. Oysters, clams, and mussels were first collected on the beaches near the Fields Point sewer outlet and later at various other localities in the river and bay. In the examination, inoculations were made from the liquor contained between the shells, from the contents of the intestines, stomach, and rectum, and in some cases from portions of the visceral mass. In order to obtain samples of the juice from an oyster under aseptic conditions, the specimens to be examined were scrubbed thoroughly in tap water with a stiff brush, washed off in running sterile water, and dried on a sterile towel, after which they were opened with a sterile knife. To obtain cultures from the stomach, the top of the mantle covering the anterior end of the oyster was slit open and the large palps on either side of the mouth pushed aside; the mouth region was sterilized by passing a hot scalpel over these parts and a portion of the stomach contents was drawn out by means of a fine pipette or platinum loop introduced through the mouth opening. Cultures from the intestines were made in the following manner: After opening the shell, the oyster was removed from the shell and dried between filter papers. A hot spatula was then passed upon the surface of the mollusk directly over that portion of the intestine which it was desired to reach, and the tube was then opened with a sterile scalpel. Through this opening a portion of the contents was drawn out by means of a pipette or platinum loop. Portions of the visceral mass were obtained by cutting out cubes of flesh from that portion of the body after sterilizing the surface with a hot scalpel.

The samples thus obtained were subjected to the same tests that were used in the water analysis—the dextrose fermentation, litmus lactose agar, and carbol broth. In these tests a nutrient gelatin medium containing 0.05 per cent carbolic acid was also employed.

Results.—The first specimens examined were oysters from Fields Point. They were collected at low tide in about 2 feet of water on the long flats that make out from the southern shore of the point. Though live material was scarce near shore, large numbers of dead shells were everywhere scattered over the flats at a little distance from land, and when the oysters obtained from this locality were opened they were found to be lean and unhealthy. The bodies were dark brown, almost black in color, while the mantle folds were, in 8 of the 10 examined, a bright green color.

There are also some clam flats and thatch grass, in which mussels were found, on the south shore of the point, within half a mile of the sewer outlet. A good set of clams was found in this beach in 1900, and at the time these specimens were secured several diggers were rapidly filling baskets for the market. These clams were large and fat, though the shells were black, and the "rims" and "snouts" were dark yellow in color. There were, however, large numbers of dead clams strewn everywhere over the beach, and a drift of white shells marked the high-tide limit. When these clams were dug samples of the sand were also taken 6 inches below the surface. A few mussels also were obtained from the thatch near by. Perhaps it may be repeated that the beach on the southern exposure of Fields Point, for a distance of more than a quarter of a mile from the sewer outlet, is covered with foul-smelling grayish slime. The water that comes up on the flats with the rising tide is charged with sewage matters, and leaves a deposit of slime on the rocks and shore below the low-water mark and a trail of filth and organic refuse along the high-water line.

The results of the analysis of these shellfish are given in the following table:

TABLE VIII.—*Analysis of oysters, clams, and mussels, collected at Fields Point.*

Specimens, and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, March 18, 1901.				
1. Juice	+	+	+	+
2. Juice	+	+	+	+
3. Juice	+	+	+	+
4. Juice	+	+	+	....
5. Juice	+	+	+	+
6. Juice	+	+	+	+
1. Intestine	+	(a)	+	....
2. Intestine	+	+	—	+
3. Intestine	+	+	+	+
4. Intestine	+	+	+	....
5. Intestine	+	(a)	+	+
6. Intestine	+	(a)	+	....
Mud on oyster shells	+	(a)	+	....
Mud on oyster shells	+	(a)	—	....
				+
Oysters, March 25, 1901.				
1. Juice	+	(a)	+	....
1. Intestine	+	(a)	+	+
2. Intestine	+	(a)	+	+
3. Intestine	+	(a)	+	....
4. Intestine	+	+	+	....
1. Stomach	(b)	....	....	....
2. Stomach	+	+	+	....
3. Stomach	+	(a)	+	....
Rectum	+	+	+	....
Clams, March 18.				
1. Juice	+	(a)	+	....
2. Juice	+	(a)	+	....
1. Pieces of visceral mass	+	+	+	....
2. Pieces of visceral mass	+	+	+	+
3. Pieces of visceral mass	+	+	+	+
4. Pieces of visceral mass	+	+	+	+
Sand	+	(a)	+	....
Sand	+	+	+	....
Sand	+	+	+	....
Clams, March 21.				
1. Juice	+	+	+	....
1. Pieces of visceral mass	+	(a)	+	....
2. Pieces of visceral mass	+	(a)	+	....
3. Pieces of visceral mass	+	(a)	+	....
4. Pieces of visceral mass	+	+	+	....
Mussels, March 21.				
1. Juice	+	+	+	....
2. Juice	—	+	—	....
1. Pieces of visceral mass	+	(a)	+	....
2. Pieces of visceral mass	+	(a)	+	....
3. Pieces of visceral mass	+	(a)	+	....
4. Pieces of visceral mass	+	(a)	+	....

[a] Not made.

[b] No growth.

Table VIII shows clearly that shellfish living in close proximity to this large sewer outlet are almost without exception infected with

Bacillus coli and other sewage bacteria. In the above recorded 10 oysters, *B. coli* was found without exception in tests made from the juice. In only one instance was it absent from the intestines, and when growth developed in tubes inoculated from the stomach content this organism was also recognized. The analysis of clams and mussels gave similar results. *B. coli* was repeatedly found in the cultures. In addition, *B. cloacæ*, *Bact. lactis-aerogenes* and *B. sporogenes* were isolated from plates inoculated with material from both oysters and clams obtained from the neighborhood of Fields Point.

The next specimens tested for *B. coli* were some clams and mussels from Pawtuxet Neck, 2 miles below Fields Point, taken on the beach which forms the northern shore of the neck where it juts out from the main shore line. The shore at this point was obviously contaminated, and only a few small clams were found alive, while the beach was strewn with heaps of the dead shells. Mussels also grew sparingly in the thatch near by. A fresh set of young oysters was observed on the piles of the Rhode Island Yacht Club boathouse, but they were too small to be of use in this work. The results of the analysis of the few clams and mussels obtained from Pawtuxet Neck are found in Table IX.

TABLE IX.—*Analysis of clams and mussels from Pawtuxet Neck.*

Specimens and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Clams, May 7.				
1. Juice	+	(*a*)	+	....
2. Juice	+	(*a*)	+	....
3. Juice	+	(*a*)	−	....
1. Pieces of visceral mass	+	(*a*)	+	....
2. Pieces of visceral mass	+	(*a*)	+	....
3. Pieces of visceral mass	+	(*a*)	−	....
4. Pieces of visceral mass	+	(*a*)	−	....
5. Pieces of visceral mass	+	+	+	....
6. Pieces of visceral mass	+	+	−	....
Mussels, May 7.				
1. Juice	+	(*a*)	+	....
2. Juice	+	(*a*)	+	....
3. Juice	+	(*a*)	+	....
1. Pieces of visceral mass	+	+	+	....
2. Pieces of visceral mass	+	+	+	....
3. Pieces of visceral mass	+	+	+	....
4. Pieces of visceral mass	+	−	−	....

[a] Not made.

Practically every specimen collected on the Pawtuxet shore contained *B. coli*. No other species of fecal bacteria were isolated.

The Sabins Point oyster ground lies directly across the river from Pawtuxet, close to the eastern shore. In April and May, 1901, two batches of oysters were dredged from this bed in from 6 to 12 feet of water on the east side of the ship channel, and inoculations were made from the juice, stomach, and intestines of these specimens. The results of the tests are given below:

Fourteen of the twenty oysters taken from this bed, 2 miles below

Fields Point, contained *B. coli*, either in the juice or in the intestines. In one case the stomach was found sterile; in another, the stomach tests did not give the reactions for this organism; in a third, however, *B. coli* was isolated from tubes inoculated with material from the stomach. The bacillus was not found in the rectum of the one oyster examined in this respect. *Bact. lactis aerogenes* and *B. sporogenes* were observed in the tests from the juice and intestines of a number of the specimens.

The majority of the oysters, clams, and mussels taken from the Providence River at a distance of about 2 miles from the sewer outlet contain evidences of sewage pollution. As might be expected, however, fewer samples from the eastern side of the river than from the western were found infected. About 70 per cent of the oysters from the Sabins Point bed contained *B. coli*, while practically all of the clams and mussels from Pawtuxet were contaminated.

The next batch of oysters examined was obtained from the Bullock Neck ground off the eastern shore of the river, not far from the Bullock Point light, about 4 miles below Fields Point. These oysters were dredged in from 18 to 20 feet of water and were obtained fresh from the boats working over the beds. Three lots were taken from this locality; in all 15 oysters were examined. Table XI is a record of this analysis.

TABLE X.—*Analysis of oysters from Sabins Point beds.*

Specimens and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, April 19, 12 feet of water.				
1. Juice	+	+	+	....
2. Juice	+	+	+	+
3. Juice	+	+	+	....
1. Intestine	+	+	+	....
2. Intestine	+	+	+	+
3. Intestine	−	+	−	....
4. Intestine	−	−	−	....
5. Intestine	−	+	−	....
6. Intestine	+	−	−	....
7. Intestine	+	+	+	....
8. Intestine	+	+	+	....
9. Intestine	+	+	+	....
10. Intestine	+	+	+	....
Oysters, May 20.				
1. Juice	+	(a)	+	....
2. Juice	+	(a)	+	....
3. Juice	+	(a)	+	+
4. Juice	+	(a)	+	....
1. Intestine	+	(a)	+	....
2. Intestine	+	(a)	+	....
3. Intestine	+	(a)	+	....
4. Intestine	+	+	+	....
5. Intestine	+	+	+	+
6. Intestine	−	+	−	....
7. Intestine	+	+	+	....
8. Intestine	−	−	−	....
9. Intestine	+	+	+	....
10. Intestine	+	+	+	....
1. Stomach	No growth.	....	....	....
2. Stomach	−	−	−	....
3. Stomach	+	+	+	....
Rectum	−	−	−	....

[a] Not made.

TABLE XI.—*Analysis of oysters from the Bullock Neck oyster beds.*

Specimens and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, October 17, 1901.				
1. Juice	+	(*a*)	−	....
2. Juice	+	(*a*)	+	....
3. Juice	+	(*a*)	−	....
4. Juice	+	(*a*)	+	....
5. Juice	+	(*a*)	+	....
1. Intestine	+	(*a*)	−	....
2. Intestine	+	(*a*)	−	....
3. Intestine	−	(*a*)	−	....
4. Intestine	+	(*a*)	+	....
5. Intestine	+	(*a*)	−	....
Oysters, October 23.				
1. Juice	+	+	+	....
2. Juice	+	+	+	....
3. Juice	+	+	+	....
4. Juice	+	+	−	....
5. Juice	−	+	−	....
1. Intestine	+	+	−	....
2. Intestine	−	+	−	....
3. Intestine	+	+	+	....
4. Intestine	−	+	−	....
5. Intestine	+	+	−	....
Oysters, October 30.				
1. Juice	+	+	−	....
2. Juice	+	−	−	....
3. Juice	+	+	−	....
4. Juice	+	+	+	....
5. Juice	+	+	−	....
1. Intestine	+	+	+	....
2. Intestine	−	−	−	....
3. Intestine	−	+	−	....
4. Intestine	+	−	−	....
5. Intestine	+	−	−	....

a Not made.

B. coli was found in a very large number of oysters, clams, and mussels taken above Bullock Neck, while only 8 of the 15 specimens taken from this (Bullock Point) bed were found to be infected. The organism was occasionally found in the juice of oysters whose intestines did not contain it. While nearly all the shellfish from Fields Point and Pawtuxet contained *B. coli* and other fecal bacteria, and while 70 per cent of the oysters from the Sabins Point bed were found to be infected, about 53 per cent of the specimens taken from the Bullock Point bed, 4 miles below the main sewer outlet, contained the colon bacillus.

The next lot of oysters was obtained from the bed off the northern shore of Conimicut Point, which, as already stated, is 5½ miles below Fields Point and is planted in from 6 to 18 feet of water. Twenty-five oysters from this bed were opened and examined, with the following results (p. 224), cultures being taken from the intestines only of these specimens.

TABLE XII.—*Analysis of oysters from Conimicut Point.*

Specimens and date of collection.	Dextrose.	B. coli.	Other fecal bacteria.
Oysters, May 29, 1901.			
1. Intestine	+	+	+
2. Intestine	+	+	....
3. Intestine	−	−	....
4. Intestine	+	−	....
5. Intestine	−	−	....
6. Intestine	−	−	....
7. Intestine	−	−	....
8. Intestine	−	−	....
9. Intestine	−	−	....
10. Intestine	−	−	....
11. Intestine	+	+	....
12. Intestine	+	+	....
13. Intestine	+	−	....
14. Intestine	−	−	....
15. Intestine	+	−	....
Oysters, October 1, 1901.			
1. Intestine	−	−	....
2. Intestine	−	−	....
3. Intestine	−	−	....
4. Intestine	+	−	....
5. Intestine	+	+	....
6. Intestine	−	−	....
7. Intestine	+	+	....
8. Intestine	−	−	....
9. Intestine	−	−	....
10. Intestine	+	+	+

Eight of the 25 oysters from the Conimicut Point bed or 32 per cent, were infected with *B. coli*, and *Bact. lactis-aerogenes* was found in three of the specimens.

Returning to the eastern shore, a number of oysters were collected on the layings directly south of Nayatt Point, at about the same distance from the Fields Point sewer as those taken from the Conimicut side. The channel keeps well over to the eastern shore at this point in the river, and consequently the Nayatt beds are planted in about 20 feet of water and in the course of a much stronger tide than that which sweeps the Conimicut shore. Fewer oysters from this bed were found to be infected with sewage bacteria than from any of the beds previously examined. The accompanying table gives the results of these analyses:

TABLE XIII.—*Analysis of oysters from the Nayatt beds.*

Specimens and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, May 30.				
1. Intestine	+	(a)	+	....
2. Intestine	−	(a)	−	....
3. Intestine	−	(a)	−	....
4. Intestine	−	(a)	−	....
5. Intestine	−	(a)	−	....
1. Juice	+	(a)	+	....
2. Juice	−	(a)	−	....
3. Juice	+	(a)	−	....

[a] Not made.

Table XIII.—*Analysis of oysters from the Nayatt beds*—Continued.

Specimens and date of collection.	Dextrose, fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, October 14.				
1. Intestine	+	+	+	....
2. Intestine	+	+	+	....
3. Intestine	−	−	−	....
4. Intestine	−	+	−	....
5. Intestine	−	−	−	....
6. Intestine	−	+	−	....
1. Rectum	−	−	−	....
2. Rectum	+	−	−	....
Oysters, October 22.				
1. Intestine	+	+	+	....
2. Intestine	−	−	−	....
3. Intestine	−	+	−	....
4. Intestine	−	−	−	....
5. Intestine	+	+	+	....
Oysters, November 5.				
1. Intestine	−	+	−	....
2. Intestine	−	−	−	....
3. Intestine	+	−	−	....
4. Intestine	−	+	−	....
5. Intestine	−	+	−	....
1. Juice	+	−	−	....
2. Juice	−	+	−	....
3. Juice	+	+	−	....
4. Juice	−	+	−	....

These results show a still further decrease in the infection. About 23 per cent of the specimens examined contained *B. coli*. It is possible, however, to demonstrate the presence of sewage bacteria in oysters living from 5 to 6 miles distant from the sewer outfall.

Passing now from the western side of the river below Conimicut Point, a number of samples—four batches, making 32 oysters in all—were taken from the Rocky Point oyster ground. Two batches were obtained from the beds off Bayside, and two from the beds off Warwick Neck, below Rocky Point. No one of the tubes inoculated with material from the intestines of these specimens gave reactions for *B. coli*. This organism was, however, found in the juice of a single oyster from the Bayside beds. The stomachs of 10 of the specimens were examined, but *B. coli* was not found in any of these analyses. These lots were taken from the river at a distance of 6 and 7½ miles, respectively, from the Fields Point sewer outfall.

Oysters from the Warren River grounds, on the other hand, were more or less contaminated by sewage. Table XIV gives the results of the analyses of 8 oysters dredged off the mouth of this river.

TABLE XIV.—*Analysis of oysters from Warren River.*

Specimens and date of collection.	Dextrose, fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, October, 1902.				
1. Juice	+	+	+	....
3. Juice	+	+	+	....
4. Juice	+	+	+	....
5. Juice	+	+	+	....
6. Juice	+	+	+	....
1. Intestine	+	+	+	....
2. Intestine	−	−	−	....
3. Intestine	+	+	+	....
4. Intestine	+	+	+	....
5. Intestine	+	+	+	....
6. Intestine	+	+	+	....
7. Intestine	+	+	−	....
8. Intestine	−	−	−	....
5. Stomach	(*a*)	....	....	....
6. Stomach	+	−	−	....
8. Stomach	−	−	−	....

[a] No growth.

The intestines of 5 of the 8 oysters examined contained *B. coli*, which was also found in the juice of these 5 specimens. The stomachs of 3 were tested for colon forms, and two tests gave negative reactions; the tube inoculated from the third oyster remained sterile.

The next lot of oysters examined was obtained from the beds situated under the lee of Pine Hill Point, Prudence Island. These beds are 12 miles from Fields Point, out of the direct course of the river, which is continuous with the eastern passage of the bay, and are farther removed from any source of sewage pollution than are any other oyster beds in the river or bay. The intestines of 10 specimens from this bed did not contain colon forms. The juice and stomachs of 5 were found to be free from any sewage bacteria.

A more extended study was made of the oysters from Wickford Harbor, which, as has already been stated, is well down the western passage of the bay and far removed from sewage pollution. The water over these oyster beds has been analyzed a number of times, and *B. coli* has never been found. About 30 oysters were obtained from this locality in March and April, 1902, and examined by the fermentation-tube methods already described. No bacteria resembling organisms of the colon group were found in the intestines, though organisms fermenting dextrose broth were occasionally observed. No growth developed in 70 per cent of the tubes inoculated with the stomach content, and when growth occurred it was not due to the colon bacillus. The juice of 8 of these oysters did not contain *B. coli.*

In addition to the above series of fermentation tests for *B. coli* on Wickford oysters, a second series, with a gelatin medium containing 0.05 per cent carbolic acid, was carried out on another lot from this same locality. The intestinal content only of the oysters was subjected to analysis; no tests were made for the juice or stomach content.

After inoculation the plates were allowed to develop three or four days at room temperature and then examined for growths of *B. coli*. The oysters used were received in the laboratory twice a week during October, November, and December of 1902, and the specimens were opened within eight hours after they had been taken from the water. The intestines of 200 oysters were examined in this manner. After a week's growth all but 3 of the 200 plates remained sterile. The colonies developing on these 3 were those of a large spore-forming aerobic bacillus, which resembled *B. vulgatus* very closely in cultural features and bore no resemblance to *B. coli*. Some time after these experiments were carried on a series of control plates (nutrient gelatin containing 0.05 per cent carbolic acid) were inoculated with a known culture of *B. coli*, and it was found that this organism grew readily in the carbol gelatin.

The two remaining beds visited in the course of this work are situated, one in the entrance to Mount Hope Bay off Bristol Ferry, and the other in the Kickemuit River. One examination was made in October, 1902, of the oysters from the bed at the entrance to Mount Hope Bay. The results of this work are found in Table XV:

TABLE XV.—*Analysis of oysters from Bristol Ferry.*

Specimens and date of collection.	Dextrose fermented.	Red colonies on litmus lactose agar.	B. coli.	Other fecal bacteria.
Oysters, October 30, 1902.				
1. Intestine	+	+	+	....
2. Intestine	+	+	−	....
3. Intestine	−	+	−	....
4. Intestine	−	−	−	....
5. Intestine	−	−	−	....
6. Intestine	+	+	−	....
7. Intestine	−	+	−	....
8. Intestine	+	−	−	....
9. Intestine	−	+	−	....
10. Intestine	−	−	−	....
1. Juice	+	+	+	....
2. Juice	+	+	+	....
3. Juice	−	+	−	....
4. Juice	−	−	−	....
5. Juice	−	−	−	....

B. coli was found in the juice of but two specimens and in the intestines of a single one. The Kickemuit River beds are 4½ and the Bristol Ferry beds 7 miles from Fall River. Neither ground is contaminated by sewage from that city, and the slight pollution found at Bristol Ferry is due to local causes. Four batches of oysters were obtained from Kickemuit River. In all, 20 oysters were examined, and it was found that *B. coli* was not present in the intestines or juice of these specimens.

The foregoing analyses demonstrate the following facts:

Oysters, clams, and mussels taken from the Providence River or its shores within half a mile of the Fields Point sewer outlet contain *B. coli* and other fecal bacteria within their shells.

Practically all of the clams and mussels analyzed as representing the condition of shellfish on the Pawtuxet shore, 2 miles below the city sewer, were infected with colon bacilli; but 70 per cent, however, of the oysters taken on the Sabins Point oyster ground, which lies directly across the river from Pawtuxet Neck, were thus infected.

Fifty-three per cent of the oysters collected from the Bullock Neck layings, about 4 miles south of Fields Point, contained *B. coli.*

Thirty-two per cent of the oysters obtained from the Conimicut Point ground, 1½ miles below the locality where the oysters from the Bullock Neck layings were dredged, contained colon bacilli.

Twenty-three per cent of the specimens dredged on the Nayatt Point oyster beds were infected.

On the other hand, oysters from the Rocky Point ground, 6 to 8 miles below the chief source of sewage contamination of the river, are practically free from pollution. *B. coli* was isolated from but one specimen of a lot of 32 oysters which were obtained from these grounds.

A sharp rise in the percentage of oysters infected with sewage forms was noticed when specimens from the Warren River were analyzed. Five out of a total of 8 oysters examined were infected with *B. coli.*

Oysters from Prudence Island and Wickford Harbor do not contain *B. coli* or other sewage bacteria.

Oysters from the Kickemuit River were not infected with these organisms. Only a small percentage of the specimens taken from the layings in the entrance of Mount Hope Bay contain any trace of sewage bacteria.

COMPARISON OF RESULTS OF WATER ANALYSIS AND SHELLFISH ANALYSIS.

If we consider the presence of *B. coli* in waters and food stuffs an indication of sewage contamination, we may trace the distribution of sewage in the Providence River and Narragansett Bay as follows: Starting in the neighborhood of Fields Point and proceeding gradually down the river to the bay below, we find that all water samples taken within a radius of one-half to three-quarters of a mile from the Providence city sewer outlet contain *B. coli*, and often other species of bacteria commonly found in sewage. *B. coli* was abundant, not only in the water about Fields Point, but was readily isolated from samples of sand taken from the beaches near by; also oysters collected from these highly polluted waters, and clams and mussels from the shores within half a mile from the sewer outlet, without exception, contained *B. coli*, and in many cases other sewage bacteria, within their shells.

Nearly all the water samples collected at Pawtuxet Neck, about 2 miles below Fields Point, were found to contain *B. coli;* also most of the shellfish (clams and mussels) obtained from this section of the river were infected. Seventy-four per cent of the water samples taken over the Sabins Point oyster ground, directly across the river from Pawtuxet, gave positive tests for *B. coli*. Seventy per cent of the oysters from this ground contained this organism within their shells.

Fifty-nine per cent of the water samples taken over the Bullock Neck oyster beds, 2 miles below Sabins Point, contained *B. coli*. This organism was isolated from 53 per cent of the oysters obtained from this locality.

Fifty per cent of the water samples collected on the Conimicut Point oyster beds, but only 32 per cent of the oysters from this source contained *B. coli*.

Off Nayatt Point, 5½ miles south of Fields Point, the water is much freer from sewage pollution. Thirty-one per cent of the water samples and only 23 per cent of the oysters taken from this part of the river contained colon bacilli.

The Warren River, however, is a polluted stream, *B. coli* being frequently found in a series of samples taken at intervals from the mouth of this river to the town of Warren; and also in a sample taken in the Providence River in the flow of the tide from the Warren River, though this pollution is soon swallowed up in the larger volume of the Providence River, so that no trace of *B. coli* can be found 2 miles distant from the entrance of the Warren River. The bacillus was found in over 60 per cent of the oysters taken from the Warren River beds.

On the western side of the river, 6 to 8 miles below the sewer outlet, *B. coli* is found only occasionally and then on a falling tide. It was present in only one oyster from this section of the river.

From the above data it may be noted that the zone of sewage pollution of the Providence River reaches southward from the Fields Point sewer outlet for a distance of about 6 miles.

In Narragansett Bay proper a different set of conditions exists. The western passage is free from sewage pollution, and neither the water nor oysters at Prudence Island or Wickford are infected with the colon or other sewage bacteria.

The Fall River sewer is, of course, the principal source of contamination of the waters of Mount Hope Bay, but it is at least 4 miles away from the nearest oyster bed, and the water and oysters from the Kickemuit River are not found to be infected with any sewage bacteria. In the sample from the Narrows, the entrance to Mount Hope Bay, *B. coli* was found in a single instance. Two oysters from the beds situated off the shore of Bristol Ferry were infected.

The above results are condensed in the following table:

TABLE XVI.—*Correlation of the results of water and shellfish analyses.*

Locality.	Distance from the Providence sewer outlet.	*B. coli* in water.	*B. coli* in oysters.	*B. coli* in clams.	*B. coli* in mussels.
Providence River:	*Miles.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Fields Point	$\frac{1}{4}$–$\frac{1}{2}$	100	100	100	100
Pawtuxet	2	100	....	66⅔	75
Sabins Point oyster bed	2	74	70	....	....
Bullock Point bed	4	59	53	....	....
Conimicut Point	5¼	50	32	....	....
Nayatt Point	5¼	31	23	....	....
(Warren River)	....	100	67½	....	....
Bayside	6½	6⅔	3½	....	....
Warwick Neck	7½	0	0	....	....
Narragansett Bay:					
Prudence Island	12	0	0	....	....
Wickford	19	0	0	0	0
Mount Hope Bay (Fall River sewer):					
Kickemuit River	4	0	0	....	....
Bristol Ferry	7	20	20	....	....

THE BACTERIOLOGY OF OYSTERS FROM UNPOLLUTED SOURCES.

Before commencing the systematic examination of shellfish from different sections of the bay for sewage contamination an attempt was made to gain some knowledge of the bacterial content of oysters from sources known to be free from all sewage contamination. Inoculations were made from the juice, intestines, and stomachs of these specimens, nutrient gelatin, reaction 1.5+, being used in this work. Plates were allowed to develop for two or three days at room temperature, and the colonies were fished out and studied in pure culture. As many as possible of these cultures were identified, and a few which did not appear to be identical with species already described, yet were frequently found in the oysters examined, are described in this paper. For convenience I have prepared the accompanying chart, similar to one proposed by the American Public Health Association for use in the description of water bacteria, and have relied principally upon the list of reactions given in this table for the description of these forms. Gelatin plate cultures were also made from water samples collected at the same places from which the oysters were obtained, in order to make a comparison between the bacterial content of the oyster and the water in which it lives.

For this purpose oysters and water samples were collected at Kickemuit River, Wickford Harbor, and the shores of Greenwich Bay. Twenty young native oysters growing on a mud flat left uncovered at low water were obtained from the last-named locality. Plates were inoculated with scrapings from the stomachs of 5 of these specimens and with samples of juice from 20.

Of the 5 plates inoculated with material taken from the stomach, 3 remained sterile and 2 developed but a few scattered colonies, which, with a single exception, proved to be growths of micrococci. One of these; a yellowish growth, was a large sarcina, forming regular packets of cells, and coinciding closely with the description given for *Sarcina subflava.* A flesh-colored growth proved to be *Micrococcus carneus. M. concentricus* was also observed in these plates. The single bacillus type found was *Ps. fluorescens.*

Plates inoculated with the juice of these oysters, on the other hand, exhibited a considerable difference in appearance from those already described. An abundant growth developed in this series of plates, and in some cases the gelatin was entirely liquefied by the large numbers of bacteria present, so that only very small quantities of this juice could be added to the culture tubes. The predominant forms found in these plates were bacilli. Only three species of micrococci were observed. *M. luteus* and *M. carneus* were found in 5 out of 20 plates. A large micrococcus, forming a thick white layer on agar and agreeing closely with *M. simplex*, was found in two plates. Ten species of bacillus tpye were distinguished, perhaps the most frequently observed form being *Ps. fluorescens*, which was found in 80 per cent of the samples examined. A nonliquefying fluorescent bacillus, probably *B. rugosus*, was found in 9 plates. For the rest, *B. limosus* was found in 11 plates out of the 20 examined; a large granular bacterium which grew into long anthrax-like chains and formed small oval spores, *Bact. maritimum*, in 7; *B. vulgatus* in 5; *B. sublanatus* in 4; *B. circulans* in 7; *B. cuticularis* in 3, and *B. cyanogens* in 2 plates of the 20 examined. Most of the organisms liquefy gelatin rapidly, so that the plates are pitted with shallow crater-like depressions in two days. The same organisms were also found in a set of gelatin plates made from water samples obtained from this locality at high tide.

The Kickemuit River oyster bed furnished the next supply of oysters used in this analysis, full-grown specimens dredged in about 16 feet of water; 30 oysters from these layings were obtained and examined in the fall of 1900. The stomach content of 20 of these, samples of the juice of 15, and portions of the intestinal content of 10 were inoculated into the usual gelatin medium. The plates inoculated from the juice of these oysters did not develop growth different in many respects from that obtained from the Greenwich Bay oysters. Liquefying organisms were most numerous, often destroying the plates in a few days. Four species of micrococci were observed in this series: *M. auriantiaca*, *M. concentricus*, *M. luteus*, and *Sar. lutea.*

M. auriantiaca was found in 20 per cent of the samples examined; *M. luteus*, *M. concentricus*, and *Sar. lutea* a less number of times. The bacillus forms observed most frequently were those common in water; *B. subtilis*, *B. limosus*, and *Ps. fluorescens* were most plentiful. *Bact. maritimum* and *B. vulgatus* were also found in these samples.

Sixty per cent of the plates inoculated with material from the stomachs of Kickemuit River oysters remained sterile. Two of the 8 plates that showed growth in three days contained large numbers of colonies of *Ps. fluorescens*, *M. luteus*, *M. flavus*, *M. carneus*, and a species of sarcina (not described in this paper). A nonliquefying fluorescent bacillus was also observed in a number of cases, forming regular glistening colonies that look like small drops of water on the surface of the gelatin, which takes on a pale green fluorescence. This organism is described on the chart as bacillus No. 11.

The 10 plates inoculated from the intestinal content of these specimens developed abundant growth in two days. Liquefying bacilli were present in great numbers. Again *Ps. fluorescens* was met with in a large percentage of plates examined; also a small motile liquefying organism, bacillus No. 6, was found in 6 of the 10 plates. Colonies liquefy slowly and form bluish-white depressions in the gelatin, some reaching a diameter of 5 mm. in four days. Microscopically they have a granular center around which is a clear hyaline area that usually has a distinctly wavy margin. By transmitted light they resemble a bluish star with a dark white center surrounded by a thin, irregular bluish growth. Agar, gelatin, and broth cultures of this organism assume a characteristic brown color after about two or three weeks' growth at room temperature. Colonies of *B. mesentericus* (variety *fuscus*), *B. subtilis*, *Bact. maritimum*, and of the nonliquefying fluorescent bacillus No. 11, already referred to, were found in these plates. *M. flavus* and *M. auriantiaca* were met with on several occasions.

A more extended study of the flora of the oysters' intestines was made on several lots of specimens obtained from Wickford Harbor in the fall of 1902. In this series of experiments material from the intestinal content of 100 oysters was inoculated into the usual gelatin medium. This analysis was begun October 14, and from that date specimens were obtained twice a week for over two months. Arrangements were made with the parties controlling the Wickford oyster beds, by which oysters caught Tuesday and Thursday mornings were shipped to Providence and received in the laboratory the same day they were taken from the water. They were then immediately opened and cultures taken from the intestines.

Plates made from Wickford oysters as a rule developed a large number of liquefying colonies, and though the organisms most frequently observed were rod forms, colonies of micrococci were occasionally met with. *M. flavus* was found in 5 per cent of the plates examined; also cultures of *M. luteus* and *M. auriantiaca* were taken from colonies developing on 3 of these plates. Another coccus form more frequently met with in this examination is referred to on the chart as micrococcus No. 1. It occurs in pairs and short chains of 4 or 6 elements that vary considerably in size according to the medium on which they are cultivated. Grown on gelatin the cocci are considerably over 1 micron in diameter; in bouillon they are somewhat less than 1 micron. This organism was observed in 15 per cent of the oysters examined.

There is a greater variety among the rod forms isolated from the plates. An organism closely resembling *Ps. fluorescens* was found in 89 of the 100 samples examined. Another fluorescent bacillus occurring in 60 per cent of these plates is referred to on the chart as No. 11. This is a nonliquefying, strongly fluorescent organism that differs from No. 5 only in certain of its cultural features. These three fluorescent bacilli were found repeatedly in the plates made from the Wickford oysters. Some plates appeared to contain almost pure cultures of *Ps. fluorescens* and *B. rugosus*. Bacillus No. 2 was found in the intestinal content of 15 of these oysters. It is a small, actively motile bacillus that grows well at room temperature and at 37° C. The presence of a very dilute solution of carbolic acid in the culture medium (one drop of a 5 per cent solution to 10 c.c. of medium) entirely inhibits the growth of this organism. Four unidentified species (No. 6) already described among the bacterial flora of the Kickemuit River oysters (No. 7, No. 8, and No. 10) were found in the plates inoculated from the intestinal content of Wickford oysters. Bacillus No. 6 was found in 30 plates, No. 7 in 20 plates, and the other two in a much less number. *B. subtilis*, *B. vulgatus*, and *B. mesentericus fuscus* were isolated from the intestines of these oysters.

In a word, the bacteria living in oysters taken fresh from pure water are common water forms. An analysis of the juice of oysters is practically the analysis of the water in which the oysters live. The stomachs of 60 per cent of the specimens examined appeared to be sterile—at least no growth developed in plates inoculated with material from this organ. Most of the bacteria found in the stomachs proved to be micrococci. On the other hand an abundant growth appeared on plates inoculated with material taken from the intestines of oysters collected in different sections of the bay. Liquefying organisms seemed to predominate, and large numbers of fluorescent bacilli were repeatedly observed, but no bacteria in any way resembling sewage forms were found. For a complete list of the bacteria isolated from these oysters the reader is referred to the accompanying chart.

Bacteria found in normal oysters.

No.	Source.	Name.	Morphology.							Cultural features.												Biochemical features.														
										Broth.			Agar.			Gelatin stab.			Potato.					Liquefaction.			Gas production.					Milk.				
			Bacillus.	Micrococcus.	Diameter more than 1 μ.	United in chains.	Motile.	Forms spores.	Stains by Gram's method.	Turbid.	Scum.	Sediment.	Dull.	Wrinkled.	Characteristic growth on gelatin plate.	Deep funnel.	Surface growth.	Needle growth.	Visible.	Luxuriant.	Grows in closed arm of fermentation tube.	Grows at 37.5° C.	Facultative aerobic.	Gelatin.	Casein.	Blood serum.	Dextrose broth.	Lactose broth.	Saccharose.	Indol produced.	Nitrates reduced.	Coagulated.	Reaction acid.	Fecal odor.	Chromogenic.	Fluorescent.
1	Intestine	M	−	+	+	+	−	−	+	+	−	+	−	−	−	−	+	+	−	−	....	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−
2	do	B	+	−	−	+	+	−	−	+	+	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−	−	−	−
3	do	*Ps.* fluorescens	+	−	−	+	+	−	−	+	+	+	−	−	−	−	+	+	+	−	....	+	+	+	+	−	−	−	−	−	−	+	−	−	−	+
4	do	B. vulgatus	+	−	+	+	+	+	+	+	+	..	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−
5	do	B. rugosus	+	−	−	+	+	−	−	+	+	+	−	−	−	−	+	+	+	+	....	−	+	−	−	−	−	−	−	−	−	+	−	−	−	+
6	do	B	+	−	−	−	+	−	−	+	+	+	−	−	−	+	+	+	−	−	+	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−
7	do	B	+	−	+	+	+	+	+	−	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−
8	do	B	+	−	−	−	+	−	+	+	−	+	−	−	−	−	−	−	+	+	−	−	−	+	+	+	..	..	..	−	−	+	−	−	−	−
9	do	M. flavus	−	+	−	−	−	−	+	+	−	−	−	−	−	−	+	+	+	+	−	+	−	+	−	−	−	−	−	−	−	+	+	−	+	−
10	do	B	+	−	−	+	+	+	+	+	..	..	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	..	..	−	−	+	−	−	−	−
11	do	B	+	−	−	−	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	+
12	Juice	Bact. maritimum	+	−	+	+	−	+	?	−	+	+	−	−	+	−	+	+	+	+	?	+	−	+	?		−	−	−	−	..	..	..	−	−	−
13	do	B. limosus	+	−	+	+	+	+	?	+	+	+	−	−	+	+	−	−	−	−	−	−	−	+		+	−	−	−	−	..	..	..	−	−	−
14	do	B. cuticularis	+	−	−	+	+	−	?	+	−	+	−	−	−	−	+	+	+	+	....	+	−	+	..	−	−	−	−	−	..	..	..	−	+	−
15	do	B. circulans	+	−	−	+	+	+	−	+	−	+	−	−	−	−	+	+	+	−	....	+	+	+	+	+	−	−	−	−	+	+	+	−	−	−
16	do	M. luteus	−	+	−	−	−	−	?	−	−	+	−	−	−	−	+	+	+	−	....	−	−	+	..	..	..	..	..	..	..	+	+	−	+	−
17	do	M. carneus	−	+	−	−	−	−	+	−	−	+	−	−	−	−	+	+	+	−	....	+	+	+	−	−	−	−	−	..	..	−	+	−	+	−
18	do	B. sublanatus	+	−	−	−	+	+	−	+	−	−	−	−	−	−	+	+	−	−	....	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
19	do	B. mesentericus var. fuscus	+	−	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	..	−	−	−	−	−
20	do	B. subtilis	+	−	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+	+	−	+	−	+	+	..	−	−	−	−	..	+	−	−	−	−
21	do	B. cyanogens	+	−	−	+	+	−	?	+	+	−	−	−	+	−	+	+	+	−	+	+	+	+	−	−	−	−	..	..	+	−	−	−	−	−
22	do	Sar. lutea	−	+	−	−	−	−	?	−	−	+	−	−	−	+	+	+	+	+	?	+	−	+	−	−	−	−	−	−	−	+	−	+	+	−
23	do	Sar. subflava	−	+	−	−	−	−	?	−	+	+	−	−	−	−	+	−	−	−	....	−	−	+	−	−	−	−	−	−	−	−	+	−	+	−
24	do	M. auriantiaca	−	+	+	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	....	+	−	−	−	−	−	−	−	−	−	..	..	−	+	−

ANALYSIS OF OYSTERS FROM UNPOLLUTED SOURCES WHICH HAVE BEEN PLACED FOR A TIME IN POLLUTED WATER.

In connection with the above work, it has been interesting to note the effect produced on uncontaminated oysters by allowing them to stand for a time in water highly charged with sewage matters. A number of the oyster companies controlling beds in the lower river and bay have docks and opening houses bordering on the Seekonk River, in the neighborhood of the outlet of one of the small sewers draining the east side of the city of Providence. At times the water in the vicinity of these wharves is filled with all sorts of organic refuse, which passes down the river in a slow stream by the docks. After the oysters have been dredged in the river below, they are brought immediately to the city, and are very often dumped into shallow cars moored close by the oyster houses, where they are allowed to remain in the filthy river water until the openers are in need of new material. They often remain in these cars from one to three days, and thus have plenty of time to take in a good supply of sewage bacteria, even though they were not infected when first brought to the city.

Two batches of oysters that had lain in floats in the Seekonk River for a time were subjected to the same tests used in this work on fresh material. One batch of 5, dredged off Warwick Neck, had lain sixteen hours in one of these floats. As has already been shown, Warwick Neck oysters are not infected with *B. coli* or other sewage bacteria when taken from the beds, but the juice of these 5 specimens gave positive reactions for this organism; it was found in the intestines of 2 or 3 examined. The other batch was brought up from the Kickemuit River, whose beds also are free from sewage pollution and are not infected with the colon bacillus. After these oysters had lain in the car for two days, however, this organism was readily isolated from the juice of all 5, though it was not found in the intestines of any of them. Water samples taken in these cars also contained *B. coli.*

CONCLUSIONS.

The sewage-contaminated area of the Providence River extends downstream from the outlet of the city sewer at Fields Point, a distance of about 5 miles. Below this area is a section about 2 miles wide, extending from one side of the river to the other, in which *B. coli* is occasionally found. The tides and wind have considerable effect upon this section, since evidence of sewage pollution has been found only when samples were collected at very nearly low water.

The waters of Providence River and Narragansett Bay from localities more than 8 miles distant from the principal sewers that discharge into this basin do not contain sewage matters, and do not give positive tests for *B. coli.*

The waters of Mount Hope Bay, at least in the areas occupied by oyster ground, are also free from sewage pollution of any extent.

The Warren River, however, from the town of Warren to its union with the Providence River, must be considered a polluted stream.

Examination of oysters and other shellfish from various portions of the river and bay show that there is a distinct relation between the presence of *B. coli* in the water and in the shellfish living in these waters. When *B. coli* is entirely absent from the water it can not be found in the shellfish, but when the surrounding waters are infected with it it is almost certain to be found in the shellfish. It seems probable, however, that *B. coli* may be present in larger numbers in the water than in oysters, for almost without exception a much larger number of water samples than oysters from a given locality gave positive reactions for sewage bacteria. This fact may be explained as due partly to the influence of the tide currents and partly to the resistance against infection exerted by the oyster itself.

Examination of the shellfish from the lower river and bay demonstrate that the bacteria usually occurring in oysters taken from uncontaminated waters are such forms as are commonly found in water. No organisms of the colon group were isolated from these oysters. Hence, analysis of the juice contained within the shells of oysters, clams, and mussels is practically an analysis of the water in which these molluscs are living. The stomachs of oysters are often found sterile. *B. coli* has been found in the juice of oysters whose intestines were apparently free from infection.

No organism which will grow in the presence of 0.05 per cent of phenol has been found in oysters from an unpolluted source.

The results obtained in these experiments indicate that *B. coli* is not normally found in sea water or in the common edible shellfish, and that the presence of this organism in oysters, clams, mussels, and similar shellfish is an indication of sewage pollution.

BIBLIOGRAPHY.

CAMERON, Sir CHARLES. On Sewage in Oysters. British Medical Journal, 1880, vol. II, p. 471.

KLEIN, E. Oysters and Typhoid. Twenty-fourth Annual Report of the Local Government Board, 1894–95.

BULSTRODE, H. T. Oyster Culture in Relation to Disease. Twenty-fourth Annual Report Local Government Board, 1894–95.

CONN, H. W. Oyster Epidemic of Typhoid Fever at Wesleyan University. Medical Record, 1894, vol. 46, p. 743.

FOOTE, C. J. A Bacteriological Study of Oysters with Special Reference to them as a Source of Typhoid Infection. Medical News, 1895, vol. 66, p. 320.

JOHNSTON-LAVIS, H. J. The Possible Conveyance of certain water-borne Diseases, especially Typhoid Fever, by Oysters and other Molluscs. British Medical Journal, 1895, vol. I, p. 559.

DE GIAXA. Oysters and Typhoid. British Medical Journal, 1895, vol. I, p. 390.

Broadbent, Sir William. A note on the Transmission of the Infection of Typhoid Fever by Oysters. British Medical Journal, 1895, vol. 1, p. 61.

Newsholme, A. Shellfish and Enteric Fever. British Medical Journal, 1895, vol. 1, p. 1285.

Eade, Sir Peter. Typhoid Fever and Oysters and other Molluscs. British Medical Journal, 1895, vol. 1, p. 121.

Wilson, W. Oysters and Typhoid Fever in Italy. British Medical Journal, 1895, vol. 1, p. 391.

Chantemesse, A. Transmission de la fièvre Typhoide par les Huîtres. Bulletin de l'Academie de Medecine, 1896, p. 534.

Wood, G. E. C. Circumstances under which infectious diseases may be conveyed by Shellfish, with especial reference to Oysters. British Medical Journal, 1896, vol. II, p. 759.

Sabatier, Ad., Ducamp, A., and Petit, J. M. Étude des Huîtres de Cette au point de vue des Microbes Pathogènes. Comptes Rendus des Séances, 125, 1897, p. 685.

Russell, H. L. Untersuchungen über in Golf von Neopel lebende Backterien. Zeitschrift für Hygiene B. XI, 1892, p. 165.

Russell, H. L. Bacterial Flora of the Atlantic Ocean, Woods Hole. Botanical Gazette, 1893, p. 383

Herdman, W. A., and Boyce, R. Oysters and Disease. Lancashire Sea-Fisheries Memoir, No. 1, 1899.

Newman, G. Oysters and Bacteria. Bacteria (Progressive Science Series) 1899, p. 231.

Kenneth, A. Oysters and Typhoid. Public Health, Vol. XXV, 1899, p. 154.

Mosny. Maladies Provoques par l'Ingestion des Mollusques. Revue d'Hygiene, 21, 1899–1900.

Ford, W. M. Varieties of Colon Bacilli Isolated from Man and Animals. Public Health, Vol. XXVI, 1900, 303.

Plowright. Clams and Enteric Fever. British Medical Journal, 1900, vol. II, p. 681.

Annual Report of the Rhode Island Commissioners of Shell Fisheries, 1900.

Philadelphia Medical Journal, 1900, Vol. VI. Typhoid from Mussels, p. 684.

Klein, E. Infection of Mussels and Cockles with the Typhoid Bacillus and the Cholera Vibrio. Twenty-ninth Annual Report Local Government Board, 1901, p. 564.

Hill, H. W. Examination of Charles River Clams. Twenty-ninth Annual Report Boston City Health Department, 1901, p. 84.

Harrington, C. Some Suspected Cases of Typhoid attributed to Oysters. Boston Medical and Surgical Journal, Vol. CXLIV, 1901, p. 439.

Bolley, H. L. An Apparatus for Taking Samples of Well Water. Centralblatt für Bakteriologie, B. XXII, p. 288.

Hill, H. W. Survival of *B. typhosus* in the Bodies of Cooked Shellfish. Journal Massachusetts Association Boards of Health, Vol. XI, No. 3, p. 96.

Klein, E. Preliminary Report on Cockles as Agents of Infectious Diseases. Twenty-ninth Annual Report Local Government Board. London, 1901, p. 574.

Marvel, P. Report on Typhoid Fever at Atlantic City. Philadelphia Medical Journal, November 1, 1902, p. 634.

Thresh, J. C. Report of an Outbreak of Typhoid Fever and other Illnesses due to Oysters. Lancet, December 6, 1902, p. 1567.

Bulstrode, H. T. "Oyster Epidemic." Mayoral Banquets at Winchester and Southampton, England, in November, 1902. Report to Local Government Board, May, 1903.

Hewlett, R. T. Note on Absence of *B. coli*, etc., from the Normal Oyster. British Medical Journal, 1903, vol. I, p. 1082.

CLARK and GAGE. Note on Colon in Shellfish. Paper read before the American Public Health Association, October, 1903.

KLEIN, E. The Bacterioscopic Diagnosis of Sewage Pollution of Shellfish. British Medical Journal, 1903, vol. I, p. 417.

FRASER, A. MEARNS. Shellfish and Typhoid. Lancet, 1903, Vol. I, p. 183.

NEWSHOLME, A. The Spread of Enteric Fever and other forms of Illness by Sewage-polluted Shellfish. British Medical Journal, 1903, vol. II, p. 295.

NASH, J. T. C. River and Shore Pollution as it affects Shellfish. British Medical Journal, 1903, vol. II, p. 297.

DOANE, R. W. Oysters and Shellfish as a Source of Disease. Pacific Fisherman, Vol. II, No. 2, p. 12, 1904.

Fourth Report of the Royal Commission on Sewage Disposal, London, England. Pollution of Tidal Waters with Special Reference to Contamination of Shellfish. Vol. I, 1904.

THE BACTERIOLOGY OF DIPHTHERIA.

By F. P. GORHAM.

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THE BACTERIOLOGY OF DIPHTHERIA.

By FREDERIC P. GORHAM,

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I. Introduction. In all the history of bacteriology, indeed of all science, there is no better example of the contribution of laboratory research to practical medicine than in the case of diphtheria. As a result of direct laboratory experiment we have gained first, an accurate method of diagnosing the disease, second, a reasonable explanation of the pathological changes and characteristic symptoms of the disease, and third, a certain cure.

Although these practical results have been actually accomplished, yet there still remain, perhaps, in the minds of many, objections to accepting some of the conclusions to which laboratory workers have been forced and so it is my purpose to explain some of these conclusions and to give the reasons for accepting them.

In the case of diphtheria as in the case of most other bacterial diseases, various organisms were at first mistaken for the true cause of the disease. On an examination of the false membrane a great variety of bacterial species was found to be present and, while we know now that this variety of forms follows in the wake of the organism which first causes the formation of the false membrane and sometimes occurs in such abundance that the presence of the true diphtheria bacillus is entirely masked, yet it is not strange but rather to be expected that in those early days other forms of bacteria were mistaken for the true cause of the disease.

The first account of the bacillus which we now know to be the cause of the disease was given by Klebs in 1883. He made no cultures but described the organism as it appeared in the false membrane. The following year, 1884, the organism was cultivated by Loeffler and he first published its cultural characters and its effect on animals. The organism has since been known as the Klebs-Loeffler bacillus.

Even Loeffler hesitated to conclude definitely that this organism was the cause of the diseases though he was able to produce the false membrane by the inoculation of his cultures, yet he did not find the bacillus in all cases examined, he was not able to produce the paralytic symptoms in animals, and further he obtained the same organism in the throat of a healthy child. Since Loeffler's first publication, however, many brilliant researches have been directed toward this organism and though we frequently hear the same objections which

Loeffler urged, yet now they can all be met satisfactorily as I shall attempt to show.

II. Is the Klebs-Loeffler Bacillus the Cause of Diphtheria? The evidence that the Klebs-Loeffler bacillus is the cause of diphtheria will be taken up under the following heads:—

1. Is the Klebs-Loeffler bacillus always present in diphtheria?

2. Is it ever present in healthy individuals?

3. Will it produce on inoculation into animals or man the symptoms of the disease?

4. The evidence from antitoxin.

1. Is the Klebs-Loeffler bacillus always present in diphtheria? There is no organism which has been the subject of so much routine examination as the Klebs-Loeffler bacillus. An enormous mass of evidence is at hand to show that this bacillus is the only species regularly present in diphtheria and that it is always present in true diphtheria. A certain small percentage of cases, it is true, fail to show it on first culture, but this is always due to imperfect swabbing of the throat or culture tube, or to the presence of other more rapidly growing organisms which crowd out the diphtheria organism in the culture tube, or perhaps to the use of antiseptic washes just previous to the taking of the culture. It can be confidently said, and the opinion of all who are competent to judge is unanimous on the subject, that there is never a case of diphtheria without the Klebs-Loeffler bacillus being present. It is most abundant at the height of the disease and disappears more or less quickly during convalescence.

Moreover, the evidence obtained from the examination of the throats of those exposed to the disease shows that the presence of the Klebs-Loeffler bacillus is in direct proportion to the immediateness of the contact. In mothers and nurses it is more likely to be found than in brothers or sisters or playmates. Park found the bacillus in about fifty per cent. of those exposed in New York, Chapin in about sixteen per cent. in Providence and Denny in about thirteen per cent. in Brookline.

2. Is it ever present in healthy individuals? The extensive investigations of the Committee of the Massachusetts Association of Boards of Health have given us abundant data for the discussion of this question.* The fact that diphtheria bacilli may and do remain in the throats and noses of patients for a longer or shorter time after recovery from all clinical evidence of disease and that the bacilli may be found in persons perfectly well, is entirely in harmony with our knowledge of the relation of disease germs to persons liable to their attacks. These germs may not necessarily be non-virulent. The fact that the person does not have the clinical symptoms is simply an expression of the fact that at that particular time he is immune to the poison produced by the germs. In convalescents, where immunity has become established, as evidenced by the very fact of recovery, we would expect that the bacilli would remain in the throat for long periods. They find favorable conditions for their growth and multiplication and there is no reason why they should become extinct, unless it is true that the immune host reacts in some way or other upon the parasitic organism.

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*Journal of the Massachusetts Association of Boards of Health, 1902, xii, 74.

The fact that well persons sometimes carry virulent Klebs-Loeffler bacilli in their throats can only be met by the statement that certain individuals have a natural immunity to the disease. The frequency with which diphtheria bacilli are found in healthy throats varies greatly. Among the well-to-do it is very uncommon unless they have recently been exposed to the disease. Among those living in crowded tenements or in children's institutions it is more likely to be found. The results of the Massachusetts committee show that something over one per cent. of healthy individuals carry Klebs-Loeffler bacilli in their noses or throats and that about seventeen per cent. of this one or two per cent. are virulent. This does not necessarily mean that the others would not regain their virulence if transplanted to other persons' throats.

3. Will the Klebs-Loeffler bacillus when inoculated into animals or into man produce the symptoms of the disease? In animals artificially inoculated with the Klebs-Loeffler bacillus the lesions resemble those seen in the human subject. They consist in a local infection about the point of inoculation, the formation of a false membrane on mucous surfaces, and a general toxemia which soon results in death. Horses, rabbits, Guinea pigs, kittens, dogs, goats cattle, hens, pigeons and small birds are susceptible, rats and mice are immune If the bacilli are introduced into the trachea of animals, a typical false membrane is formed, the lymphatic glands are enlarged and a diphtheritic palsy sometimes follows. The general picture resembles pretty closely that of laryngeal diphtheria. Roux and Yersin were the first to observe that in some cases paralysis may appear before death.

There are instances on record of the inoculation of human beings with cultures of the Klebs–Loeffler bacillus. McFarland reports as follows: "All skepticism of the specificity of the diphtheria bacillus on my part was dispelled by an accidental infection that once kept me housed for three weeks. Without having been previouly exposed to diphtheria I was one day experimenting in the laboratory and by accident a living virulent culture of the diphtheria bacillus drawn into a pipette entered my mouth. Through carelessness no precautions were taken to prevent serious consequences and two days later my throat was filled with typical pseudomembrane which private and Health Board bacteriologic examinations showed to contain pure cultures of the Klebs-Loeffler bacillus. This occurrence has been reported by Riesman."*

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*McFarland's Textbook upon the Pathogenic Bacteria, 1903, p. 426.

Furthermore the toxin or poisonous substance produced by the bacilli while growing in the culture tube will after being separated from the bacilli themselves, on injection into animals, produce inflammation and hemorrhagic edemas, necrosis or sloughing of tissues, and paralysis, exactly simulating the changes which occur in ordinary clinical diphtheria. This observation in itself is one of the most formidable arguments as to the causal relation of the bacillus and the disease.

4. Evidence from Antitoxin. The observations in regard to antitoxin production and its preventative and curative effects in diphtheria give still further confirmatory evidence. The fact that the proper treatment of animals with the bacilli or their toxins, will cause them to elaborate a substance which acts *specifically* in the prevention or the cure of the disease, gives us overwhelming proof that this bacillus is the real cause of the disease.

III. THE MORPHOLOGY OF THE KLEBS–LOEFFLER BACILLUS. One objection always urged against the specificity of the Klebs–Loeffler bacillus is the fact of its variable morphology and the existence of what is known as the pseudo-diphtheria bacillus. It is true the Klebs–Loeffler bacillus is variable in form. But we know that this is true of very many of the so-called species of bacteria. Under the variable conditions of culture, under the influence of the continually changing properties of the body fluids upon which it lives, is it not strange that the bacillus should retain a constant morphology and physiology rather than that it should vary? We should expect that the gradual assumption of immunity to diphtheria toxin by an individual would react upon the organism forming that toxin, and that there should be a gradual change in morphology from the type prevalent during the height of the disease and through the various stages of convalescence. This has been observed.

Furthermore we should expect that under certain conditions of manipulation or of unfavorable environment the virulence of an organism as well as its morphology should be altered and that we should find types of the bacillus which are non-virulent. My belief is that the pseudo-diphtheria bacillus is but an attenuated or non-virulent form of the Klebs–Loeffler bacillus. Others think that the two organisms are different and until some one succeeds in transforming one into the other this matter is one of opinion. The transfer of the virulent Klebs–Loeffler bacillus into a non-virulent one is possible, and has been frequently done, but the reverse process has never been accomplished to the satisfaction of those supporting the theory of the non-identity of the two organisms.

The question of the origin of the Klebs–Loeffler bacillus is perhaps pertinent in this connection. Has it always existed as a species by itself or did it arise by the assumption of virulence from some previously non-pathogenic saprophyte? To this question of course we can give no answer. It is the same question as that of the origin of all forms of living things, and although we may have theories that at some time in the earth's history an ordinary putrefactive bacterium gradually assumed the power of living as a parasite on animal mucous membranes, and that under the favorable conditions there repsented began the manufacture of a poisonous product which reacted upon its host, yet all this is but guesswork and of no value as evidence as to the real conditions which brought into existence the pathogenic bacteria.

IV. The Distribution of the Klebs-Loeffler Bacillus. We have already discussed the presence of the bacillus in the sick, in convalescents, in those more or less intimately exposed to the disease and in normal well persons. Does the organism exist elsewhere ? We might expect to find the bacillus in hospital wards and in rooms occupied by cases of diphtheria, and on the clothing and other objects used by diphtheria patients. Hill* has shown that it is possible to demonstrate the presence of the organism in some 1.8 per cent. of the examinations made of the bed clothing of patients sick with the diphtheria; in 0.86 per cent. of the examinations of floors and furniture in the sick room; and in 15.3 per cent. of the examinations of playthings, clothing, and handkerchiefs of sick persons. But he says, "taking four weeks as the average duration of a case from earliest symptoms to release, a very large proportion of the bacilli thrown out during the first week and reported on exposed surfaces will have died from drying before disinfection is done. A large proportion of those thrown out the second week will also be dead. Even those of the third week will have suffered some weakening. There will be comparatively few organisms left on the exposed surfaces which gaseous disinfectants will reach at the end of the fourth week for the disinfectant to kill. These will be found principally on the bed clothing and body clothing of the patient. * * * * It would seem that the evidence for the necessity for surface disinfection after diphtheria is very slight." That the organism lives anywhere for any length of time outside of the animal body except in cultures in the laboratory we have no evidence at all.

V. The Diagnostic Value oe the Klebs-Loeffler Bacillus. From what we have already seen the only sure method of diagnosing diphtheria is by the demonstration of the presence of the Klebs-Loeffler bacillus. Whenever the laboratory finding coincides with the clinical diagnosis no trouble arises. If, on the other hand a positive report is made on a patient whose clinical symptoms are not those which the physician is accustomed to regard as diphtheria, or on a well person, the physician may unwillingly yield to the result or discredit it entirely. But it must always be remembered that the bacteria are not the immediate cause of the disease. The diphtheria bacillus produces disease only by means of the poisons which it manufactures, and although the bacillus may be present in a patient, yet it may not be secreting poison, or that poison may be neutralized by the immunity of the patient. On the other hand these same bacilli, if transferred to the throat of another individual, may become virulent, or that person may not be immune to the poison. A person who is shown by bacteriological examination to be carrying Klebs-Loeffler bacilli is dangerous to the community and the report of a positive bacteriological examination must be interpreted in this light. A negative report, however, is not good evidence of the absence of the bacilli. The first, second, even the third culture may fail to find the bacilli for reasons already stated. The clinical diagnosis, therefore, must not be given up until four or five negative cultures have failed to establish the presence of the bacilli.

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*Public Health, Papers and Reports, 1903, xxviii, 209.

THE GAS DISEASE OF FISHES.

By M. C. MARSH and F. P. GORHAM.

Appendix to the Report of the Commissioner of Fisheries to the
Secretary of Commerce and Labor for the year ending
June 30, 1904.

DEPARTMENT OF COMMERCE AND LABOR
BUREAU OF FISHERIES
GEORGE M. BOWERS, Commissioner

THE GAS DISEASE IN FISHES

BY

M. C. MARSH
Assistant, Bureau of Fisheries

AND

F. P. GORHAM
Associate Professor of Biology, Brown University

APPENDIX TO THE REPORT OF THE COMMISSIONER
OF FISHERIES TO THE SECRETARY OF COMMERCE
AND LABOR FOR THE YEAR ENDING JUNE 30, 1904

Pages 343 to 376. Plates I to III

WASHINGTON
GOVERNMENT PRINTING OFFICE
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THE GAS DISEASE IN FISHES.

By M. C. Marsh, *Assistant, Bureau of Fisheries*, and F. P. Gorham, *Associate Professor of Biology, Brown University.*

INTRODUCTION.

The pathologic symptoms and changes which affect fishes and some other aquatic animals, and are here grouped as a unity under the general term "gas disease", do not include all abnormal manifestations of gas or symptoms involving gas. In the literature of the pathology of the lower animals gas disease does not appear to be recognized definitively, though some of the numerous references to gaseous symptoms, and particularly to the so-called "pop-eye" in fishes, doubtless apply to the disease as here discussed and limited. In cattle and other mammals certain bacterial diseases are accompanied by evolution of gas within the tissues.

The gas disease of aquatic animals was first observed and recognized among fishes in sea water at the station of the Bureau of Fisheries at Woods Hole, Mass. It has been observed also at other stations, at the New York Aquarium, and among fresh-water fishes, and it has without much doubt occurred at private establishments. At Woods Hole it is known to have progressed for several years.

In general terms the cause of the gas disease lies in the relation of the amount of air dissolved in the water in which the affected fishes live to temperature and pressure. An understanding of this relation will follow a consideration of the laws of solubility of gases in water and particularly of the gases which constitute the atmosphere.

SOLUBILITY OF GASES IN WATER.

The amount of a given gas which pure water will dissolve depends upon temperature and pressure and upon the solubility of the gas. Under increased pressure the capacity of water for holding any gas is increased, while at an increased temperature this capacity is diminished. The coefficient of solubility varies widely for the different gases. Oxygen and nitrogen, which chiefly make up the atmosphere, are but slightly soluble in water, while carbon dioxid, which contributes a very small part to the total atmospheric bulk, is extremely soluble.

Ignoring its minor constituents and regarding the argon group of gases with the nitrogen, the atmosphere is approximately made up of 79 parts of nitrogen gas and 21 parts of oxygen gas by volume. The carbon dioxid present has no particular connection with the gas disease and will not be referred to further. When water is exposed to the atmosphere it absorbs these two gases until a state of equilibrium is reached, when no further change takes place and these gases, if the temperature and pressure remain constant, are neither further absorbed nor given off by the water. The latter is then said to be saturated with air. If now any change takes place in the temperature of the water, or in the pressure which it sustains, either a further absorption will occur or some of the air will be given off from the water. These changes, especially under artificial conditions, may occur rapidly, and the adjustment to an equilibrium may not keep pace; therefore, at at any given time water may fall short of saturation and air be passing into it, or it may be supersaturated and air be passing away from it, assuming of course in either case that it is not protected from contact with the atmosphere. In other words, water may hold in solution an excess or a deficiency of air, or an excess or deficiency of either one of the air gases, nitrogen or oxygen, independently of the other. The rapidity with which water supersaturated or infrasaturated with air will become saturated, or in equilibrium, will depend upon the area of its contact with the atmosphere. It therefore follows that water only moderately exposed to the atmosphere, as in tanks or most containers, may remain for a considerable time either above or below the saturation point. But the tendency is constantly toward the equilibrium of the saturation point, which will always finally be reached.

The actual amounts of nitrogen and of oxygen which water will absorb from the atmosphere have been determined by analyses of air-saturated water. Authorities differ somewhat in the results. The figures cited here and in the tables give the highest values. One liter of pure water at 0° C., the freezing point, and at a pressure of 760 mm. of mercury, the standard atmospheric pressure, will absorb 19.53 c. c. of nitrogen from the atmosphere (Pettersson and Sonden) and 10.18 c. c. of oxygen (Winkler); at 20° C. and 760 mm., 12.8 c. c. nitrogen (Dittmar), and 6.35 c. c. of oxygen (Winkler). Pure sea water takes up somewhat less. These figures are taken from Comey's Dictionary of Solubilities.

RESPIRATORY PROCESSES AND MECHANISM IN FISHES.

To understand the effect of supersaturated water upon fishes it is necessary to consider the respiratory processes and the mechanism by which a dissolved gas could gain access to their circulation. In warm-blooded animals the life processes depend upon the absorption of oxygen by the tissues and the elimination of carbon dioxid, and this interchange is effected through the medium of the blood. The liquid portion of the blood, the plasma, carries but a small portion of the total oxygen dissolved in the blood. This portion is in amount about what an equivalent volume of water would absorb, and is held in simple solution, as in water (Foster, 1895, p. 588). Most of the oxygen of the blood is carried by the red corpuscles, which are vehicles for this gas by virtue of the hemoglobin they contain, with which oxygen readily combines and from which it may readily be separated. The tissues of the body have a stronger affinity for the oxygen than that which exists between the hemoglobin and the oxygen, and they therefore take the oxygen from the hemoglobin of the corpuscle, and give in return carbonic acid, not to the corpuscle, but to the plasma of the blood. When the blood next reaches the lungs it gives up this carbonic acid to the external air, while the hemoglobin of the corpuscle takes up a new supply of oxygen from the air. Though the blood does not come into direct contact with the atmosphere, the corpuscles come into intimate relation with it and are separated from it only by a thin layer of epithelial cells, constituting the final subdivision of the lung. Through this membranous partition the interchange of gases takes place by diffusion, the process being known as osmosis, and the permeable membrane as an osmotic membrane. Osmosis is governed by laws analogous to those of simple diffusion of gases, or of the absorption of gases by liquids, and depends therefore in part on the pressure exerted by each gas concerned. The blood side of the membrane is high in carbon dioxid and low in oxygen, while the air side is high in oxygen and low in carbon dioxid. Each gas exerts its pressure independently of the other, the carbon dioxid to pass out toward the air, the oxygen to pass in toward the blood. The tendency is to equalize each gas on the two sides of the membrane, when the pressure on both sides would be equal and osmosis would cease. Since in life this can never occur, because the carbon dioxid going out is continuously produced within and the oxygen coming in is continuously used up within, there is a continuous stream of these two gases passing in different directions, and at an osmotic pressure which does not vary greatly under usual conditions. Any increase of the proportion of oxygen in the atmosphere, or any increase of barometric pressure, would increase the osmotic pressure and more rapidly force the oxygen into the blood. The workman in the compressed-air caisson labors under a high osmotic pressure, which may seriously affect the respiratory process.

The nitrogen of the air is normally taken up by the blood in amounts insignificant as compared with the oxygen, and is held in simple solution, probably in the plasma alone. One hundred volumes of arterial blood hold some twenty volumes of oxygen, but only from one to two volumes of nitrogen (Foster, 1895, pp. 586, 601).

The physiology of respiration in cold-blooded animals is not so completely known, but the broad facts cited above apply equally to fishes. There is the interchange of oxygen and carbon dioxid, the corpuscle with hemoglobin as the carrier of the oxygen and a set of vascular filaments fulfilling the same office as the lungs. The gills are immersed in water instead of air, but this does not greatly alter the nature of the breathing process. The blood merely gives up carbon dioxid to and takes up oxygen from a solution of these gases in water instead of directly to and from an atmosphere which they partially constitute. The epithelium of the gill filament is the osmotic membrane, and in this case the osmotic pressure of the oxygen and of the nitrogen depends upon the amount of these gases in solution in the water and not directly on the atmospheric pressure, though the latter has an influence on the amount of air dissolved in the water. The nitrogen is not known to play any part in respiration and the plasma probably remains with a fairly constant quota of this gas corresponding to the amount of nitrogen dissolved in the water, which is usually air-saturated with it. In water recently boiled and containing scarcely any oxygen the osmotic pressure due to oxygen is practically nothing, and in this fishes suffocate. The highest osmotic pressure under ordinary conditions experienced by fishes occurs when water at the freezing point—or slightly colder, since salt-water fishes can live in water below 0° C.—is so well aerated that it has dissolved all the air it will hold at whatever atmospheric pressure exists. Of fishes in higher osmotic pressures than this no cases are known to the writers save those here described, and experimental observations under such conditions seem not to have been made.

SYMPTOMS AND LESIONS OF THE GAS DISEASE IN FISHES.

The occurrence in fishes of lesions of a gaseous nature is no recent observation. A certain exophthalmia known in fish-cultural parlance as "pop-eye" has long been recognized and is due in many cases to the presence of a gas either behind the eyeball or within it. This may be accompanied by inflations of the mucous membrane lining the mouth cavity or of the skin elsewhere, and these lesions may exist independently of the so-called pop-eye. At the Woods Hole station of the Bureau of Fisheries these symptoms have been observed during the summer for years among marine fishes held in aquaria for purposes of exhibition, and have been described by Gorham (1899). In very cold water at the same place, other conditions remaining the same, the course of the disease is more rapid and the symptoms somewhat different. In aquaria of sea water a few degrees above the freezing point fishes show within some three minutes after their introduction a reaction consisting of extremely minute and very close-set gas bubbles. Within about ten minutes the bubbles visibly increase in size and become much more conspicuous, enveloping the fish completely, body and fins, in a delicate, shimmering layer of silvery white. It is evident that the bubbles do not emanate from the fish itself, although they appear to; almost any surface within the water, as that of rocks and the sides of the aquarium, exhibits the same phenomenon. Neither are they free bubbles afloat in the water which happen to attach themselves by contact to the bodies of fishes—though this may occur and simulate, in any water, the appearance under discussion—because the same occurrence takes place after all free bubbles have been allowed to rise and escape and fish are immersed in perfectly clear and quiet water. The gas is a precipitate from the water itself, in which it must have been in solution. At first, while the bubbles are very small, they are quite closely adherent and the fish may execute rapid movements without dislodging them. As they grow larger they detach themselves readily and rise to dissipate at the surface. A sudden movement will release a cloud of hundreds or thousands of bubbles. A few seconds' removal of the fish from the water will completely dissipate all the bubbles, but after its return to the water they are soon formed again in their usual abundance. In fact, these bubbles are more or less a feature of all the fishes as long as the latter remain in water of this quality.

The gas in the tissues, which manifests itself in blebs of the greatest diversity in size and location, does not appear immediately, but only after several hours at the earliest. The blebs may arise at any point, the favorite seats being the fins and the head (fig. 1, pl. I). This lesion consists merely of a local accumulation of gas in or beneath the skin, the outer layer of which is often stretched to an attenuated thinness by the expansive pressure. If the so-called "slime" of the skin is abundant, bubbles may form within it, in which case they are small and numerous. The tautog has an abundance of this slime and presents a characteristic picture after a reaction of several hours. The bubbles tend to buoy the slime and tear it from the body; it is partly separated in long streamers, which remain attached at one end while they float suspended in the water, buoyed by the bubbles which cling to the surface and are embedded within the substance. This fish takes on, after about an hour, a strikingly ragged and tattered appearance, which is shown by no other species save the cunner. In fact, each species exhibits the external gaseous lesions in a way more or less peculiar to itself. The tomcod is especially prone to develop a few extraordinarily large vesicles of gas in its fin membrane. The buoyant action of these is often considerable, and when they are present in the caudal or last dorsals they tilt the fish out of position and require a constant effort to overcome their effects. The tautog, besides the appearances cited, ha almost invariably small elongate blebs between the rays of the pectora and usually also the caudal fin. The small sculpin (*Myoxocephalu æneus*) seldom fails to develop in the skin of the belly an emphysem of a honeycomb structure; and often in the later stages, by coalescenc or enlargement, vesicles containing several cubic centimeters of ga may form, floating the fish belly upward long before it finally succumbs.

Very young puffers (*Spheroides maculatus*, fig. 1, pl. II), when only half an inch long, develop vesicles at the base of the caudal fin sometimes as large as the entire body of the fish, which buoy it to the surface and keep it there in spite of its struggles to descend. The pipe-fish (*Siphostoma fuscum*) usually shows vesicles about the snout. In the scup (*Stenotomus chrysops*), both large and small (2-inch), the first indication of the presence of gas is seen in the protrusion of the eyeballs, bringing about the condition known as pop-eye.

These external lesions, however, though interesting and important in their bearing on the explanation of the disease, are not sufficient to cause death. Aside from some occasional bloody streaks in the fins, eyes, or muscles, neither constant nor characteristic, no external lesions other than these are to be found and no adequate cause of death is to be seen. It is on laying open the dead or dying specimens that the fatal lesion is disclosed. A remarkable and striking picture presents. The blood vessels contain notable quantities of free gas, the amounts varying greatly, from a few small bubbles scattered through the larger vessels to a quantity which may distend the bulbus of the heart even to several times its normal bulk, stretching its walls to a thin membrane, tense and firm with the pressure of the gas contained to the entire exclusion of the blood, the whole resembling the air bladder of a small fish. The auricle may be still beating without propelling any blood. The fish may live for some time, probably for days, even after considerable quantities of gas have separated; for upon killing and opening scup not yet in the death struggle the gas has been plainly discerned. The walls of the auricle and ventricle may be emphysematous. The branchial artery or ventral aorta is often empty of blood and tense with the pressure of gas, while in the gills is found perhaps the most constant and significant lesion. The main vessel of the gill filament usually has its lumen filled with gas (fig. 2, pl. I), which is often seen just entering the capillaries that branch from this vessel. But these capillaries it seldom fills. The gas plugs of the gill filaments are usually present—though not always—even when the evidences of gas within the body are not very marked. A fatal embolism results, and death is due to stasis.[a] When nearly all the filaments are well filled with gas the condition modifies considerably the macroscopic appearance of the gill, and in fishes of some size the individual emboli may be seen on careful inspection by the naked eye.

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[a] In these typical cases of embolized gill filaments and of a distended heart, no assumption of any form of initial cardiac paralysis seems necessary. The stasis must have occurred in spite of cardiac effort.

The gas has not been observed in the capillaries of the body, but is confined to the larger vessels of the systemic circulation and the gills. It does not distend the veins, though bubbles may be seen in them. In sculpins in full roe the arteries ramifying over the surface of the ovary attract immediate attention by their appearance as pale bloodless streaks in contrast with the green background of the ovary and the dark red of the veins which accompany them. Gas bubbles may be seen in the pyloric cœca, in the walls of the intestine, and also within the intestine itself, though these latter may be due to other causes.

CAUSE OF THE GAS DISEASE IN FISHES.

POSSIBILITY OF INFECTION BY GAS-PRODUCING BACTERIA.

The inference to which all the gas symptoms at first give rise, of infection with gas-producing species of bacteria, has been negatived by repeated attempts to obtain cultures from the blood and tissues of affected fishes, among both the Woods Hole marine forms and those of fresh water. The microscope gives no evidence of infection, and inoculated culture media remain sterile. The Woods Hole sea water suffered no unusual pollution and the bacterial count at the intake in January and February averaged only 191 per cubic centimeter. The rapidity of the pathologic process, furthermore, contraindicates infection.

ABNORMAL GAS CONTENT OF WATER IN WHICH THE DISEASE OCCURS.

The sea water in which fishes die with these described lesions always has an extraordinary gaseous content. At the Woods Hole station it had passed through a pumping plant which elevated it to storage tanks to provide a gravity flow for aquarium and hatching purposes. Steam pumps took the water from the sea through a long suction pipe and forced it to a height of about 18 feet into tanks, from which it flowed to the aquaria and hatching boxes. At the point of intake the sea water was of normal quality and fishes lived in it without unusual symptoms. The suction pipe was of wood, had been long in use, and by deterioration had developed areas of porosity or open leaks, so that air continually gained access to the pipe and could readily be demonstrated at the pump, which forced a mixture of water and large quantities of air bubbles instead of a solid body of water. Immediately upon passing the pump this air and water came into a region of about 8 pounds hydrostatic pressure in addition to that of the atmosphere, and continued under this pressure through a long stretch of level water main. As the sea water was approximately saturated with air at the intake it inevitably acquired a supersaturation on its journey from the pump to the storage tanks, due to the presence of air and the increase of pressure. In the storage tanks there was but slight exposure to the atmosphere and from them the water reached the aquaria containing its excess of air. In the aquarium tanks the water gives some evidence of its unusual condition in the form of precipitated bubbles of gas which gather on all solid surfaces in contact with the water, and in a minute effervescence which is barely visible when its perfectly smooth, unbroken surface is carefully observed. The actual effect of the release of these bubbles is to diminish but inappreciably the degree of excess while the flow is continuous, for the constant inflow is bringing new supplies of the supersaturated water.

Influence on respiration of fishes.—The gill apparatus of fishes, for the osmotic interchange of gases which keeps the blood purified, is presumably adjusted to water the gases of which were dissolved at atmospheric pressure. The gills of any fishes in this aquarium water are therefore subjected to an osmotic pressure higher than any to which they were habituated in nature. Osmosis is accelerated and the blood takes up unusual quantities of air. The goal toward which the process tends is the same degree of supersaturation on one side of the gill membrane as on the other. In other words, the osmotic pressure on the two sides tends to equalize, and, inasmuch as blood and water have approximately the same saturation point, the blood stream tends to acquire the same excess of air as the water, or to become actually supersaturated with air. This is believed to be what actually takes place. The circulation becomes supersaturated.

In cases where fishes are brought up from considerable depths and confined in this water, the great reduction of pressure acting on the gas in the air-bladder and tissues permits the expansion of this gas. There is an attempt on the part of the fish to remove this excess gas, first by absorption into the blood and second by osmosis through the gills. But the second part of the process is inhibited by the already high gas content of the water in which the fish are placed. Thus in these fishes the supersaturation of the blood is more readily brought about.

The subsequent release of gas within the vessels is to be explained chiefly by temperature changes within the blood. While fishes are cold-blooded animals, their body temperature is not exactly uniform with that of the surrounding medium. The combustion involved in the life processes implies the evolution of heat, and this heat is appreciable and has been measured. The venous circulation shows the highest temperature, and in fishes of several common marine species has been found to be from 2° to 12° F. warmer than the surrounding water (J. H. Kidder, 1879). Between the gills and the systemic veins, then, the blood undergoes a greater or less elevation of temperature, for in its course through the gills it must be cooled to or nearly to the temperature of the water. Its stream is too thin and it is too intimately exposed to the water to maintain an appreciably higher temperature. The blood, then, before it can return a second time to the gills, undergoes a rise in temperature, and as the solvent power of liquids decreases with increase of temperature, this rise tends toward the release in gaseous form of some of the dissolved air. No doubt the amount released is small per unit of time, but the free gas can not be reabsorbed and the process of release is continuous, so that a fatal embolism is only a question of time.

This seems a fairly satisfactory explanation of the means by which the gas arrives free within the blood vessels. It requires the assumption that in water of normal condition with respect to dissolved air the blood of fishes does not become completely saturated in the gills—else gas would be thrown out constantly by the higher temperature of the systemic circulation, which is of course contrary to fact. There is experimental proof that in mammals ordinary respiration does not saturate the blood—that is, that all the oxygen which it is capable of holding under the conditions does not enter it (L. Fredericq, 1896; O. Hammarsten, 1901, 531). That the observation holds good for fishes is extremely probable. It must further be assumed that under the conditions of supersaturation existing in the Woods Hole water the blood does take up all the air it will hold at its temperature in the gills; or, if it falls short of this, that it takes up more than it can hold at the maximum temperature to be encountered in its circulation through the body. This latter supposition is the more probable and, while no determinations support it, it is thoroughly in accord with the facts and may be provisionally accepted.

Temperature is not the sole cause which may play a part in the precipitation of the gas. For the separation of the solute, or dissolved substance, from a supersaturated solvent, there must be a nucleus about which the precipitating dissolved particles may gather—an excitant to start the process of precipitation. This is strikingly illustrated by supersaturated solutions of certain salts. A crystal of the same salt as that dissolved when introduced into such a solution will cause the immediate separation of this salt, which gathers about the crystal as a nucleus. Likewise water may be heated, in a perfectly clean and smooth flask, above the boiling point without ebullition. If a solid foreign particle, such as a fragment of pumice stone, be dropped into the flask, boiling instantly begins. To apply this principle to the present case, the minute floating corpuscles may be considered as the nuclei for the separation of gas from blood, which is supersaturated with it. The difference in temperature is the more important and fundamental cause of the release of gas, while doubtless the corpuscles at least provide *loci* for the change of state.

The time required for a fatal result depends primarily on the degree of excess. Death has been observed within three hours after introduction of a healthy fish into the abnormal water, but in this case the exact excess is unknown, and there was no autopsy. At 10° C. and an excess of 6 c. c. of nitrogen and 2 c. c. of oxygen per liter, a hake was killed at the end of 8 hours, and embolic gas under pressure in the heart was observed immediately after death. Ten hours frequently suffices for this result. Species differ in susceptibility.

Identity of gas in the blood vessels, external vesicles, and water.—Some relation of identity or source between the several gases within the blood vessels, in the external blebs, and that which separates directly from the water upon the fishes is at once inferred as probable, and the gas of all the lesions would seem to be derived from the water. The following four samples were determined by the United States Bureau of Chemistry, the first three collected in February. The merely adherent bubbles which formed on the exterior of the fishes had the following composition:

	Per cent.
Carbon dioxid	1.03
Oxygen	17.58
Nitrogen	81.39

A sample precipitated upon blocks of wood, no fishes being in the water, consisted of:

	Per cent.
Carbon dioxid	0.58
Oxygen	22.87
Nitrogen	76.55

The difference between the carbon dioxid and oxygen in these two samples should be referred to the respiration of the fishes, present in the first case and absent in the second. The gas from the large vesicles on the belly of the small sculpin (*Myoxocephalus aeneus*) was as follows:

	Per cent.
Carbon dioxid	3.78
Oxygen	18.09
Nitrogen	78.13

In this the oxygen is diminished and the carbon dioxid increased by oxidation of organic matter in the tissues. Methane, hydrogen, or carbon monoxid were not present in any of these samples.

A sample collected in September from the water alone showed:

	Per cent.
Carbon dioxid	0.4
Oxygen	20.6
Nitrogen	79.0

The gas is evidently nothing more than the constituents of air, the proportions varying more or less from those of the atmosphere.

The quantities actually dissolved in the water were first determined from a sealed and transported sample taken in September during the progress of the disease. The results are probably not perfectly accurate, because of the age of the sample. By reference to Table III, page 373, it is seen that this water had an excess of nitrogen of 2.2 c. c. per liter, and was a little less than saturated with oxygen. The time which elapsed between the taking and the determination of the sample, however, probably removed oxygen by oxidation, and there may have been an original excess of this gas as well as nitrogen.

Elimination of the disease by reducing gas content of water.—The replacement of the old suction pipe with a new impervious one abolished all signs of the gas disease at Woods Hole. Determinations made upon the water of the aquarium after air had been intentionally admitted to this new suction pipe showed definite and considerable excesses of both nitrogen and oxygen, these determinations being made at the station upon freshly taken samples. The gas was boiled from the water by the Tiemann and Preusse modification of Reichardt's apparatus (Hempel, 1902, p. 10) and determined by absorption, the residue after removal of carbon dioxid and oxygen being considered as nitrogen. There appeared an excess of both nitrogen and oxygen of some 3 to 6 c. c. per liter of water in the case of nitrogen, and of 1.5 to 2.5 c. c. of oxygen. This condition of the water killed 6 hake in from 8 to 20 hours. The figures are probably somewhat greater than those for the conditions of the old leaking suction pipe, which may be represented by an excess of about 2 c. c. of nitrogen per liter, and of somewhat less than 1 c. c. of oxygen. The water under the experimental conditions referred to with the new suction pipe had exactly the same effect upon fishes as the water during the service of the pervious wooden suction pipe, save that it was more rapidly fatal. The dead fishes showed all the described lesions and symptoms. It is left beyond question that the gases of the pure atmosphere are one of the efficient factors in the causation of the gas disease.

Exposure of the water to the atmosphere at atmospheric pressure removes the excess of air with a rapidity dependent on the degree of this exposure. Whenever by the mechanical arrangement of the delivery pipes at the aquaria the inflow of water was exposed, as when a strong jet was allowed to impinge upon the surface of the aquarium level, carrying in many bubbles of free air, the lesions on the fishes were more slowly produced, and the fatal result was postponed. The process of exposure deaerated the water, and had only to be made thorough enough to correct it completely by removing the excess. Thus, if the inflow was made to pass through a strainer elevated several feet above the aquaria, so that the water was divided into many very slender streams, which compelled intimate contact with the air during the drop and in the splash at the surface, all mortality and symptoms of gas could be prevented. From water standing without flow in ordinary containers the excess of course finally disappeared, but in the large Woods Hole aquaria signs of excess were still evident after seven days. A cylindrical glass hatching jar of about 2½ gallons capacity, after filling with supersaturated water, required to stand two or three days before this water failed to produce an external precipitation on the body of a tomcod immersed in it as a test.

RÔLES OF NITROGEN AND OXYGEN IN CAUSATION OF THE DISEASE.

Some consideration may now be given to the separate rôles of the two gases nitrogen and oxygen in the disease. A reference to Table II, page 373, shows that the gas from the fixed gas lesions, that is, from the exophthalmia, from the fin blebs, and particularly from the chambers of the heart, is very high in nitrogen.

The sample from the sacs of rainbow-trout fry was taken from specimens preserved in formalin and some oxygen may have been lost on this account. All the others were from fresh material.

The samples upon which these figures are based were very small, and in obtaining them it was impossible to exclude with certainty all contamination from atmospheric sources. In each case a part of the small percentage of oxygen found certainly came directly from the air. The sample from the eyes of scup was most liable to this error. That from the hearts of various fishes indicates that the gas which causes the fatal embolism in the vessels is almost pure nitrogen, and samples from this source more accurately represent the gas as released from the blood than those from the external blebs or the tissues about the eyes. The one sample of the latter sort obtained was largely from scup in which gas had inflated the conjunctiva so that this gas was separated from the water only by a very thin transparent membrane, through which oxygen from the water may have diffused. Likewise all the fin blebs have but a similar osmotic membrane protecting the contained gas from changes in its original composition. The heart gas, however, doubtless represents solely a direct precipitation from the blood. It would appear, then, that it is the nitrogen gas chiefly, if not solely, which plays the essential part in the disease. The separation of gas from the supersaturated blood is certainly not in proportions analogous to that of the separation of nitrogen and oxygen from water supersaturated with air. In air-saturated water the oxygen is about 33 per cent of the total oxygen and nitrogen dissolved. In water air-supersaturated under the mechanical conditions here described the percentage of oxygen dissolved is slightly less, for the excess is not taken up in the same proportions that it is from the atmosphere. When unsaturated water is shaken with air at ordinary pressure, the residue of undissolved gas is richer in nitrogen than the atmosphere. But in this mechanically induced supersaturation fragments of the atmosphere are forced bodily into solution in their entirety, and the dissolved content is increased by nitrogen and oxygen in atmospheric proportions, 79+21, instead of in dissolved proportions, 67+33. When the excess of these two gases escapes spontaneously from the water the oxygen has about the atmospheric relation to the nitrogen, i. e., about 21 per cent of the total, notwithstanding that while in solution the oxygen is more than 30 per cent of the total of these two. In other words the excess goes in as air and comes out as air. Thus the actual analyses already cited (p. 354) of precipitated gas from Woods Hole water, show the proportion of oxygen to be about as in air.

Since the blood does not release its supersaturation in this way, it is at once suggested that the hemoglobin capacity for oxygen modifies the effect of the water so far as the supersaturation with oxygen is concerned. It would appear that the corpuscles can take up more than the usual amount of oxygen and that the increment is not thrown out by the rise in temperature. It remains to study experimentally the effect upon fishes of water in which the supersaturation is with oxygen alone. Some evidence is afforded by an instance of such a supersaturation, naturally occurring, in a pond containing trout. At the Cold Spring Harbor Station of the New York Forest, Fish, and Game Commission, the springs which chiefly supply the station make immediately a shallow pond of considerable size. In the spring of 1904 the bottom of this pond became heavily overgrown with green algæ, chiefly with a species of *Spirogyra*. Presumably from these algæ, the water about the middle of the pond acquired an excess of oxygen of 3 c. c. per liter, while the nitrogen content remained normal, or but slightly in excess. Remote portions of the pond were normal in oxygen. Large trout lived in it in good condition and showed no gas symptoms, but the fact lacks conclusiveness since they had access to normal water, which they doubtless frequented. It is probable, however, that a large excess of oxygen is required to produce untoward results from this gas alone. In the conditions at Woods Hole, while the excess was of both oxygen and nitrogen, it is probable that the damage was done by the latter gas alone.

RELATION OF GAS DISEASE TO TEMPERATURE AND PRESSURE.

When water is here described as containing an excess of air, or an excess of oxygen or nitrogen, a definite relation of the quantity of gas to temperature and pressure is of course connoted. It is hardly necessary to insist that dissolved gas only is referred to, for loose bubbles present are not really in the water, though they may be beneath its surface or within its volume. The gas-disease process, then, bears an intimate relation to temperature and pressure. If a given quantity of dissolved air per unit of water, at a given temperature and pressure, occasions a fatal process among fishes, a sufficient increase in the pressure or decrease in the temperature may render the water perfectly harmless to fishes; but it does so by abolishing the excess of air, though no change occurs in the absolute quantity of air concerned. The temperature factor alone is not so easily varied, and no direct experiments have been made involving it, but the statement above can hardly fail to be corroborated by such tests. For the pressure factor some interesting experimental facts have been obtained.

Scup placed in live boxes at or near the top of a reservoir storage tank of the Woods Hole water which was causing gas symptoms in aquaria were usually killed within twenty-four hours, the characteristic embolism and external symptoms always present. At the bottom of this tank, the depth of water being 8 or 9 feet, several days were required to produce the symptoms, and death occurred only after a still longer time. At half the depth the results were intermediate. There was a constant flow of water through the tank and it was evidently the hydrostatic pressure which inhibited the usual process. Carrying these observations further, a large glass jar was arranged to hold aquarium water with a constant flow and under a pressure varying between 6 and 7 pounds per square inch in addition to atmospheric pressure. Five adult scup were placed in this jar and remained alive under the pressure, without food, for twenty-nine days without developing any gas symptoms. The same water which flowed through the jar would at the beginning of the experiment at atmospheric pressure produce external lesions within twenty-four hours and was fatal within two or three days, the time varying considerably. After removal of pressure at the end of the experiment, all the five scup died within five days with free gas in the vessels of each. They were fed for the first time on the fourth day after the removal of pressure. During various experiments at Woods Hole some evidence was incidentally brought out indicating that starvation retarded the gas-disease process. This it may be conceived to do by a general lowering of metabolism.

Except under experimental conditions, no cases of gas disease caused by reduction of pressure alone have been observed by the writers, and it is doubtful whether any occur. In a former paper by one of us (Gorham, 1899) it was thought that the reduction of pressure was the only cause. The factor of the supersaturation of the water was not recognized at that time. From experiments performed in connection with that former work and new ones in connection with the present study we are sure that mere reduction of hydrostatic pressure—that is, the reduction incident on bringing fishes to the surface of the water—is not sufficient to produce the disease in those fishes which have been studied. A number of scup were kept in a live car at the surface of the water outside the hatchery for twenty-four days and no symptoms of the disease appeared. At the end of this time, when placed in the supersaturated water of the aquaria, the same scup died quickly, with all the symptoms of gas disease. There is a considerable reduction of pressure brought about in bringing scup from their natural depths (2 to 20 fathoms) to the surface. The pressure at 5½ fathoms is twice that at the surface. But the fish can accommodate themselves to this reduction. The increased volume of gas in the air bladder is diminished through absorption by the blood, and the gills remove it by osmosis to the sea water.

Experimentally, however, reduction of pressure below that of the atmosphere is sufficient to produce the disease. The experiments reported in the previous paper (Gorham, 1899), which have been repeated and extended, demonstrate this. They were carried on by placing fishes in sea water in a large jar from which the air could be exhausted by a pump, and the vacuum secured measured by a gauge. Fishes could be killed very quickly (forty-four minutes) by a rapid reduction of the pressure to about 20 inches of vacuum, or about one-third of an atmosphere. These fishes gave the symptoms of gas disease, such as the presence of a gas bubble in the heart and gas in the other vessels. By a less reduction, or by a series of reductions with periods of rest between, it was possible to bring about the formation of the external lesions of the disease, such as pop-eye, blebs in the fins, etc. Similarly an increase of pressure, brought about by forcing air into this same jar or by subjecting fishes to the pressure of a considerable depth of water, will cure or prevent the disease. Symptoms of the disease such as protruding eyes and blebs on the fins, which have been caused by placing fishes in supersaturated water, will disappear when the fishes are placed under these conditions of increased pressure. It should be said, however, that the presence or absence of an air bladder is probably important in determining the presence or absence of free gas within the blood vessels of fishes drawn from depths to the surface. There seems to be no reason why such fishes lacking an air bladder should show embolic gas or any free gas which was not free at the beginning of the change of depth. As far as the writers are aware, no observations have been made or are of record. While the saturation point of both water and blood at great depths is tremendously increased, deep waters do not have a greater air content than surface waters. They have, in fact, less of oxygen, and of nitrogen approximately the same as or less than surface waters, but never more. (Dittmar, 1884, p. 225.) This follows from the fact that the air in deep waters was taken up at the surface, and that the oxygen may be constantly diminished by oxidation processes while the nitrogen remains unchanged. The blood of deep-sea fishes without air bladders should never, therefore, contain more air than it can hold at the coldest surface water. As for pressure conditions, then, no such deep-sea fish should liberate air from its blood when brought to the surface. Since, however, its habitat may be water whose nitrogen was dissolved at a low temperature, and it may be brought up into comparatively warm surface water, there exist theoretical conditions in which this result would be possible. That it actually occurs is unlikely, but is a matter for observation. The air-bladder factor has not been thoroughly worked out in the present study and is an interesting field for further experiment.

Although under these experimental conditions it is possible to produce the gas disease by reduction of pressure alone, yet the conditions are quite different from those which obtain when fishes are brought to the surface from depths. Fishes are in the habit of coming to the surface for short periods under natural conditions. They can accommodate themselves for short intervals, at least, to changes in pressure ranging from that at the surface to that of considerable depths, though the amount of gas to be eliminated when a fish with an air bladder comes to the surface is very large. When forcibly drawn up from considerable depths great changes take place, for the eyes bulge from the head and sometimes completely out of the sockets, the fish is often "poke-blown," the stomach and other viscera pushed into the cavity of the mouth, and the air bladder expanded or ruptured. The removal of pressure causes the free gas always present within the body to expand, and occasions displacement of tissues and organs. It is an interesting question whether such fishes have free gas within the blood vessels.

When the pressure is reduced below that of the surface, and quite rapidly, we would expect that the fish's powers of accommodation would be overstepped and they would not be able to take care of the surplus gas so quickly produced. No opportunity for adjustment is given. Still more when a fish is brought from a considerable depth and confined at the surface in water which is already supersaturated with gas, the gills would be unable to discharge the excess from the blood and the production of the gas disease would be hastened.

On the other hand, that the supersaturation of the water alone without the reduction of pressure is sufficient to produce the disease, we have abundant evidence. Surface fishes like *Fundulus*, usually quite hardy, succumb to the effects of the supersaturated water. Fresh-water fishes, like the trout, which have never been subjected to any decrease of pressure, quickly show the effects of supersaturation.

CONCLUSIONS.

In the light of these facts it seems to follow theoretically that no matter how great the quantity of air dissolved in water no gas disease can appear, provided the pressure is high enough; and conversely, no matter how high the pressure the gas disease will appear, provided the quantity of air dissolved is great enough. Supposing the temperature constant, it is the interrelation of the dissolved air factor and the pressure factor which determines the fact of the excess, and since the condition of excess of air is to be defined only as a preponderance of the dissolved air factor over the pressure factor, the cause of the gas disease may be defined broadly as due to an excess of air; and more narrowly, since there is much evidence that nitrogen alone is essentially concerned, as due to an excess of nitrogen.

SUPERSATURATION OF NATURAL WATERS.

The symptoms and fatality at Woods Hole were the result of artificial conditions. A modification by the hand of man of the conditions under which air is usually taken up by the water resulted in an excess of the air so taken up. The pertinent question will immediately suggest itself whether natural waters ever acquire a similar excess, or any excess at all, of air or of the constituents of air. Such excesses are found to occur. Natural springs of water and flowing wells are known to emit a gas, sometimes in considerable quantities, which has approximately the composition of air. These are not very common.

Conditions at Erwin, Tenn.—Such a spring occurs on the reservation at the Fisheries Station at Erwin, Tenn., in a limestone region near the foot of a considerable mountain ridge. This spring has a superficial area of about 600 square feet and its maximum depth is about 4 feet. The bottom is partly of mud, partly of gravel and the outcropping of the limestone strata. The water wells up chiefly from the gravel, and from each wellspring a quantity of gas in large bubbles is evolved at intervals of a few moments. The gravelly bottom about the sources of water holds mechanically large amounts of gas, for, upon tapping it gently with a stick, an unusually large quantity is liberated and comes bubbling up through the water. The evolution of gas then ceases for a longer period than usual, but begins again spontaneously within a few minutes. This periodical delivery of gas continues day and night at all seasons. Evidently there is a constant flow of gas accompanying the flow of water and at all times in the earth or gravel beneath the spring and through which the water rises are entangled large quantities of gas, a small fraction of which is evolved every few minutes as the pressure beneath determines.

This gas is air with the nitrogen and carbon dioxid considerably increased. (Table I, p. 372, sample 1.) As springs do not usually discharge both water and free air, the original access of air is of more than passing interest. It is evident that it must be mainly derived from the atmosphere.

The region about this spring is mountainous and largely of a limestone formation, in which caverns have been formed by the usual process of solution of the limestone by water containing carbon dioxid.

The surface water percolates through cavernous limestone. An aspirating effect is probably produced by the flow through fissures and narrow channels which have access to air spaces, and the air is sucked in and mingled with the down-flowing water, which it accompanies to the mouth of the spring. During the journey a diminution of the oxygen may occur from oxidation, which may reasonably explain the modified proportions of these gases. Though the mountainous region referred to abounds in springs, only a single other bearing air was found, and this a small one by the roadside.

Air-bearing springs or wells of this character are to be distinguished from the "breathing" or "blowing" wells abundant in some sections, which alternately emit and suck in air from causes among which variations in the barometer are important. In Nebraska many wells having this remarkable peculiarity occur, and have been described by the United States Geological Survey (E. H. Barbour, 1899). The springs of supersaturation which deliver bubbles of air constantly are probably unrelated to breathing wells and, as far as known, pass the air in one direction only.

The water of this Tennessee spring was apparently of excellent sanitary quality—clear, cold (about 12° C.), slightly alkaline, and contained an excess of nitrogen, but not of oxygen. It was slowly fatal to fishes placed directly within the spring. Trout fry between 1 and 2 inches in length were killed by it sometimes within a day or two, although some individuals would survive in it for weeks. On fish of this small size no internal gas within the vessels was in any case demonstrated with certainty. Neither were external symptoms usually present, but in the hatchery troughs supplied by the spring they were more frequent and sometimes extremely conspicuous, consisting of emphysema of the skin, either single cysts of gas, sometimes of relatively great size, smaller multiple cysts, or small blisters of gas, which usually had their seat upon the head or mucous membrane of the mouth cavity. Apparently the only inconvenience the fry experienced from these was a mechanical one. The buoyancy of the gas was often great enough to keep them constantly at the surface, and its unequal lateral distribution gave them a list to one side or the other. They did not appear to be materially weakened.

When older trout, yearling rainbows 6 to 8 inches in length, were introduced into this spring, symptoms more closely resembling those at Woods Hole resulted. Death occurred with moderate symptoms of external gas, with gas free in the heart, though not abundant enough to cause distention, and with emboli of gas in the gill filaments. The susceptibility of species varied widely, and gold-fish were not affected during a trial of sixteen days, while other cyprinoids succumbed almost as readily as the trout. These experiments with fishes in the spring were made in live boxes and were controlled by the same or similar boxes in the spring water after it had passed from the spring and been improved or corrected by exposure to the air, and these controls suffered no loss.

Determinations of the degree of excess of nitrogen in the Erwin water have not been made on freshly taken samples. The origin of the excess is to be looked for in the rising gas and the necessary pressure factor in the weight of the column of springing water. The air bubbles are presumably mingled with this water for a distance below the restricted areas of emergence in the spring in its subterranean course and even the whole distance back to its surface origin. The greatest depth reached by the water beneath the spring is unknown, but is estimated from the geology of the region to be at least 100 feet, and may be several hundred. This depth represents the height of the column of water, the pressure of which is operating constantly to force the air bubbles into solution. The supply of bubbles is abundant and never failing, and the water is bound to take up more air than it can hold when it reaches the surface and becomes exposed to the atmosphere at atmospheric pressure only. Here the excess begins to escape; and as the spring is shallow and well exposed, this process is rapid; yet the constant flow keeps the body of water constantly supersaturated. In flowing away from the spring in shallow exposed channels the water soon corrects itself, becoming normal and harmless to fishes. By applying devices in the hatchery, thoroughly exposing to the air the water supplying the troughs, the gas symptoms disappeared and the losses were reduced to the normal for all fish-cultural operations.

CONDITIONS AT NASHUA, N. H.

At the fisheries station at Nashua, N. H., occurred still another case of a water supply abnormal in its air content, and here an excess of nitrogen coexisted with various degrees of deficiency of oxygen. The station supply came largely from rather shallow artesian wells, some of which entered the hatchery directly, while others were driven in the bottom of the nursery and rearing ponds and on the edge of the larger brood ponds. Many field determinations of the dissolved oxygen and nitrogen in the water of the Nashua station were made and are shown in Table IV, page 374. There appears a deficiency of oxygen of greater or less degree and a moderate excess of nitrogen in the water of every source of supply save that from the taps of the Nashua city service. This latter water, however, at its source in artesian wells (Pennichuck wells) is even more abnormal as to dissolved air than is the station water, the oxygen being less, the nitrogen about the same. While not insanitary for city purposes, it would doubtless be fatal to fishes. The aeration and deaeration it receives in the open stream which takes it to the reservoir adjust these abnormalities, so that as delivered from the service pipes it has about a normal quantity of air. The same adjustment occurs with the station water after it has flowed through the hatchery troughs, ponds, etc., and has gathered in a waste brook at some distance below the hatchery and pond system, save that the process has not been complete enough to remove all the excess of nitrogen ("creek water" sample, Table IV). In fact, the adjustment begins the instant the water emerges from the wells, and in most cases by the time it reaches the fishes it contains somewhat more oxygen and less nitrogen than at the well. The effect of the deaerating process on the loss of trout fry was shown by passing the "reservoir pond water" through a finely perforated metal plate with a fall of about 3 feet. During a trial of nine days a trough containing 6,000–7,000 fry lost 645, against a loss of 2,583 in a similar trough containing the same number, but supplied directly from the pond without deaeration. The process, which did not completely correct the water, reduced the loss 75 per cent. Complete correction would probably result from repeating the process or by sufficiently increasing the fall. The water of hatchery well No. 1 was completely relieved of its excess of nitrogen by allowing it to flow drop by drop down an inclined wooden plank 10 feet in length. (See Table IV, p. 374.)

Very few of the Nashua wells delivered free gas, and these only in small amounts. From one of these about 500 c. c. were delivered and collected during twenty days and constitute sample 2 of Table I. Only air gases were present. Part of the sample was tested for methane, unsaturated hydrocarbons, and carbon monoxide, without showing a trace of any of these. (Dr. D. A. Morton, Syracuse, N. Y.) The sample had no marked odor. The largest pond at the station, used chiefly as a reservoir supply and largely spring-fed, had a soft bottom from which occasional large bubbles rose. By ramming the mud with a stick, large quantities of a gas about 96 per cent nitrogen (Table I) could be released. Methane, which might have been expected, was absent. This gas seems to be of much the same origin as that from the air-bearing spring in Tennessee, though delivered in much smaller quantities, and may reasonably be supposed to come from a depth great enough to cause the supersaturation which existed in this pond, as in all the sources of water in the vicinity.

At the Nashua Station the gas symptoms were in evidence, but were less marked than in either of the other described cases of the results of supersaturated water. Exophthalmia with presence of gas appeared in adult trout in ponds, and the general condition of these trout was poor. This condition is believed to be secondary to the supersaturation, which, while not sufficient to kill the adults directly by embolism, causes the protrusion of the eye and consequent inflammation. The partial or total blindness resulting keeps them from feeding properly, and as they fall off in condition and become weaker they are attacked by the fungus *Saprolegnia*. Among the deaths which resulted no case of free gas within the vessels was discovered. The fry showed occasional gas blisters externally, and in very young fry gas was frequently to be seen within the sac. Even in the Woods Hole water some few fishes, and many in the spring water of a lesser supersaturation, died without evidence of sufficient internal gas to produce an effectual embolism or enough apparent mechanical disturbance to account for death. Yet these fishes no doubt died of the excess of air. It is possible that in these cases there were internal lesions that escaped observation, minute emboli of gas, for instance, in the vessels of the brain, though in a number of brains examined no gas had reached their vessels. It seems probable that the metabolic functional disturbance due to the abnormal osmosis is itself sufficient to cause death without apparent gas symptoms.

EXOPHTHALMIA OR "POP-EYE."

Though not necessarily always occurring in all cases of supersaturated water, this affection is so prominent among symptoms of gas disease as to deserve special consideration. It is not an infallible sign of supersaturation. As "pop-eye" or "frog-eye" it has long been familiar to fish culturists, and these terms are vernacular for any protrusion of the eye from its orbit, whatever may be the essential cause. It is not a disease, but a symptom, the expression of any one of a variety of causes or underlying conditions. Inflammation, from a wound or other irritation within the orbital cavity, may cause a swelling of the tissues which pushes the eyeball outward from its position. Specimens of this sort are not very common in shallow natural waters. One specimen, a butter-fish (*Peprilus triacanthus*), which apparently falls in this class, was taken from the trap nets at Woods Hole August 3, 1903, and examined immediately by the writers. It showed a moderate exophthalmia on each side. The globus was still lenticular in shape, and on dissection under water no sign of gas was detected. The brain and optic nerves appeared normal. If there had been a traumatic injury evidence of it had disappeared. The inflammation was not pronounced, and while an exudate behind the eye was, in part at least, the immediate means of the displacement, the primary cause can not be given. Externally the condition simulated strongly that caused by supersaturation, to which in this case it could not possibly have been due.

Mechanical injuries alone, as a sudden blow upon the head, may produce an immediate protrusion of the eyeball (Hofer, 1904, p. 292).

Among the menhaden which died from the epidemic prevailing during the summer of 1904 in Narragansett Bay there were many cases of pop-eye, due, no doubt, to the injuries received during the peculiar death struggles characteristic of the disease. In some cases of pop-eye, where gas is plainly present and responsible for the displacement, it is possible that some other cause than supersaturation with air may be concerned, though none such is definitely known to the writers. In the great majority of cases where gas is present the cause will be found to be an excess of air, with spring waters usually an excess of the nitrogen of the air alone, and the location of the gas will be behind the eyeball. Some species of fishes are not susceptible to this symptom from supersaturated water, or at least it has not been observed in them. The anatomical structure and the degree of the excess seem to be the factors which control. Among marine fishes, the dog-fish (*Mustelus canis*) and other sharks, eels, puffers, sea-robins, the flat-fishes, and others do not develop typical cases, if any, while the scup, the king-fish (*Menticirrhus*), the tautog, the cunner, the sea bass, and the butter-fish may exhibit it in various degrees. Of all these the scup (*Stenotomus chrysops*) shows it most readily and in extreme degree (Plate III). With a certain degree of excess not exactly known, but probably above 3 c. c. of nitrogen per liter, embolism becomes fatal before there is time for an accumulation of gas behind the eye. An excess of not over 2 or 3 c. c., and probably less, per liter is favorable to the development of the symptom, which may be taken to indicate a moderate excess of air. The eyeball is sometimes pushed almost completely out of the head (Gorham 1899, Plate 12). Without much displacement of the ball the conjunctiva may be raised and inflated into a balloon of gas projecting far out beyond the eyeball (Plates I and II of this paper).

Among fresh-water fishes salmonoids chiefly have been seen to be affected. The black sucker (*Catostomus nigricans*) showed a typical case at Erwin, while some cyprinoids (*Notropis galacturus* and a *Hybognathus*) under the same conditions died with the eyes normal. It is no doubt because not many fishes save the trout of artificial propagation have been observed in supersaturated fresh water that few fresh-water species are known to show the lesion. In brook and rainbow trout the pop-eye is seldom so extreme as that shown in the illustrations of the scup. The excess being slight, the symptom may grow very slowly and be present for months, or even years, impairing more or less the activities of the fish. Blindness frequently results, with accompanying increases of dark pigment in the skin. The exposure of the eyeball makes it subject to injury, and it is sometimes bitten off by other fishes, or drops or sloughs away, leaving the socket empty.

In trout fry past the sac stage a certain exophthalmia may develop after death if they remain in water, and the younger and smaller the fry the more quickly it appears. In general its development requires from twelve hours to three days. Evidently there is a physiological post-mortem accumulation of transudate behind the eye. There is a pathologic exudate which occurs in trout fry suffering from anemia and this exudate may localize, sometimes in the abdominal cavity, causing ascites, sometimes behind the eye, causing exophthalmia without gas. Fry having this form of anemia, though their eyes still be normal at death, more readily than healthy fry develop in water the post-mortem exophthalmia which in this case seems to be partly physiologic and partly pathologic. Likewise among a brood of fry suffering constant losses from supersaturated water many of the dead will be found with a greater or less, sometimes an extreme, exophthalmia without the presence of gas. It is a post-mortem occurrence, but the previous gas disease process seems to favor its development. All these cases, however, are to be carefully distinguished from the gaseous exophthalmia, directly a symptom of the gas disease.

The source of the gas behind the eye must be taken to be the blood. Its position appears to make it impossible that it be derived directly from the water. The blisters of gas which form upon the exterior of the body and fins seem explainable as derived from either source, and whether this gas has really passed through the blood of the fish or come through the permeable integument directly from the supersaturated water can not at present be stated, but the evidence is somewhat in favor of the latter view. It is probably chiefly in the large veins that the precipitation of the embolic gas from the blood occurs. The supersaturating gas is acquired at the gills, subsequent to which there is a fall of blood pressure. These facts make it probable that the peripheral circulation is supersaturated, and that an essential condition for the precipitation of gas at the periphery is supplied, though all the causes which combine to bring the dissolved gas in the blood of the capillaries free within the tissues are not clear. On the other hand, the presence of supersaturated water on one side of the very membranous covering of the fins, and on the other side tissues bathed in a lymph, which at the beginning is not supersaturated, suggests a more immediate reaction by the ordinary laws of osmosis.

THE CAISSON DISEASE ANALOGY.

The gas disease of fishes is paralleled in man by an affection in which, so far as it holds, the analogy is striking. The compressed-air disease—caisson illness, diver's palsy, etc.—is caused by an increase of air pressure; with divers, by the weight of the water above; in the caisson, by the compression necessary to keep the water out. In so far as the subject sustains an extraordinary pressure the analogy does not hold, for the gas disease involves no necessary increase of pressure upon the fishes themselves. But the osmotic process of gases passing into the blood through the lung membranes, under compression, must be intensified according to the height of the pressure, as it is through the gill membranes, in supersaturation, according to the degree of the excess. In this and in the results the two cases are much alike. The caisson disease has long been known and has a considerable medical literature, but some uncertainty seems to have existed as to the immediate cause of the symptoms and of death. The mechanical effect of the compression was supposed to be important, but recently the influence of this factor has been pronounced nil. Bubbles of gas in the blood vessels are at the bottom of the trouble. Hill and MacLeod (1903) have the following to say:

> Paul Bert, by his remarkable experiments, published in 1878, proved that the true cause of caisson sickness is the effervescence of gas in the blood and tissue juices. * * * He found that this gas (nitrogen) was set free on rapid decompression and produced embolism in the lungs, the central nervous system, etc.; and that the gravity of the result depended on the height of the pressure, the length of exposure, and the rapidity of decompression. He also proved that the gas set free in the tissues might produce local swellings and emphysema.
>
> Bert also found that high oxygen tension acts as a general protoplasmic poison arresting metabolism, depressing the body temperature, and causing the discharge of convulsions in mammals and finally the death of all forms of life.

The following are a part of the summary by the same authors of experiments of their own:

> The cause of caisson sickness is the escape of gas bubbles in the blood vessels and tissue fluids on decompression. An animal exposed for four hours to 8 atm. air and quickly decompressed is like an opened bottle of soda water. The fluids of the body generally effervesce.
>
> The varying symptoms of caisson sickness are due to the varying seat of the air emboli.
>
> Young men escape caisson sickness owing to the elasticity of their tissues and greater facility for collateral pathways of circulation.

The effervescence of gas in the vessels of caisson workers is of course largely prevented by the precautions taken, but it is the logical result of compression followed by rapid decompression. With fishes there is, unless experimentally, no question of compression or decompression, but the gas symptoms occur under the conditions of supersaturation corresponding to compression, and no lowering or removal of supersaturation, corresponding to decompression, is necessary. The reason for this lies chiefly in the temperature factor already discussed. Theoretically the caisson worker should develop the effervescence while still under the compression, provided there is a difference of temperature between the systemic and pulmonary circulation and the exposure to compression is of long duration. This exposure is actually limited of course to a few hours at a time, and this may explain the absence of serious results during compression.

OTHER ANIMALS SUSCEPTIBLE TO GAS DISEASE.

Fishes are not the only aquatic animals susceptible to gas disease. The crustacea may survive a long time with the blood in a condition resembling foam, and in the lobster and king crab this has been readily observed through the abdominal shell. These latter usually live much longer than fishes under the same conditions of excess, but a lobster at Woods Hole was killed within thirty-six hours by an excess of about 6 c. c. of nitrogen per liter. Sea spiders (*Anoplodactylus*), as observed by Mr. L. J. Cole, are readily killed, the legs becoming filled with the gas and the color becoming much paler than in health. Mollusks, hydroids, and some green algæ also develop and emit bubbles which presumably originate in supersaturation.

EFFECT OF SUPERSATURATED WATER UPON EGGS AND FRY.

At Woods Hole sea water which was soon fatal to adults or fishes approaching maturity did not affect eggs and fry. Eggs of the cod were incubated for some two weeks in such water and the fry remained in it until planted—not more than a few days at most, it is true, but a longer period than would suffice to kill adults—yet neither were injured or showed any gas symptoms. It is probable, however, that very young fry are not necessarily immune under all conditions of supersaturation. Bubbles of gas have been noticed in the sacs of shad fry at fish cultural stations. Mr. J. N. Wisner (1900) reports such a case at Havre de Grace, Md., and the circumstances point to a leaky suction pipe, but nothing is known of the degree of supersaturation, if any existed. Theoretically it seems difficult to avoid the conclusion that oxidation must be attended by an elevation of temperature even in so minute a creature as a newly-hatched cod fry; but this elevation must be infinitesimal, for the consumption of energy necessary to maintain a temperature appreciably above the surrounding water is not supposable in the eggs or fry. As such an elevation of the blood temperature is the chief cause of gas precipitation in adults, its absence in the fry may be taken as strongly tending to explain their immunity. On the other hand, a sufficiently high degree of excess may be able to cause a separation of gas such as above noted among shad fry, either by direct osmosis or via the circulation.

METHODS OF PREVENTING THE GAS DISEASE.

The proper aeration of water, by artificial means if not already accomplished by nature, has from the beginning been recognized and insisted upon by fish culturists as of fundamental importance. By aeration was meant the process of putting the water thoroughly in contact with the atmosphere, so that the dissolved air would be increased were there any initial lack. In a proper fish-cultural sense, aeration more strictly meant oxygenation, for it was the oxygen alone, the prime necessity of fishes, which was apt to be lacking. No cases, perhaps, are known in which natural waters have less than their proper or normal amount of nitrogen. But of course the aeration process adds both the atmospheric gases should the water be lacking in both.

The readily observed distress and suffocation of fishes by the exhaustion of the dissolved oxygen in unrenewed water, the efficacy of even the simplest means of aeration in restoring the life-supporting quality to the water, as well as the generally understood necessity of oxygen to all animals, resulted naturally in an appreciation of the value and necessity of aeration. There were no observed facts from which one would infer the opposite condition in water, an excess of one or more of the air gases, nor were theoretical considerations likely to lead readily to its conjecture. It is improbable that any symptoms or mortality from this cause occur in nature, for supersaturation does not arise suddenly and aquatic animals would avoid the regions of excess in the rare cases where access to them is possible. The possibility of injurious or fatal excesses of dissolved air, especially in natural waters, seems not to have occurred either to fish culturists or biologists.

The two faults, excess and deficiency of air, are so correlated that the same process of correction applies to each. The same exposure to the air which aerates water with a deficiency of air deaerates water with an excess of air. In superaerated water, such as that of the Woods Hole aquaria, there may be a deaeration in the more complete sense; both nitrogen and oxygen are to be removed. But in hardly any case does the term aeration apply in its complete signification. Oxygenation alone is usually the strict meaning. In natural waters the term deaeration likewise does not in most cases completely apply. Denitrogenation alone is the stricter meaning. Oxygenation, however, may accompany denitrogenation, and thus water is in the broad and looser sense aerated and deaerated at the same time and by the same process.

When an actual case of air-supersaturated water confronts the fish culturist or the management of aquaria, the practical measures to be taken will suggest themselves according to the source of the excess of air. If a gravity plant supplied by pumps is in operation the whole suction system is open to suspicion of leaks. Such leaks, of course, give out no water but suck air, and are therefore not always easily recognized. By stopping the pumps and removing the proper valve the hydrostatic pressure may be allowed to rest back on the suction pipe and will speedily develop the leaks if the pipe is exposed. If it is underground they may not show readily, or at all. Repair of all the leaks will completely remedy the difficulty. The suction pipes, especially if wooden, may be beyond repair, in which case nothing but a complete renewal will entirely prevent trouble. Pending this, local deaeration may be practiced at each aquarium, pond, or trough supplied with the water. For an aquarium a large pan with many perforations may be suspended above, the higher the better, and the water delivered into this. If the exposure of the slender streams and the splashing at the surface are not sufficient correction, the scale of the device has but to be increased, most conveniently by adding more perforated pans. The great desideratum is sufficient fall in which to expose the water.

When the supersaturated supply is from springs or wells the condition is more serious. A radical correction is impossible, for the air, or modified air, which causes the excess is deep in the earth and can not be controlled. If, as is usually the case, there is no great difference of level between the rising water and the ponds, troughs, or tanks in which it is used upon fishes, it is the more difficult or impossible to completely deaerate. The natural remedy is to use the water only after it has flowed a considerable distance from its source in a shallow open stream. Failing this it may be carried through a circuit of a long and wide trough, to pass finally through perforated deaerating pans. In general a complete exposure to the atmosphere is necessary and the means for accomplishing this will vary with the conditions of each individual case. The deficit of oxygen is more readily supplied than the excess of nitrogen removed. The water eagerly takes up the oxygen it lacks, but the last traces of excess of nitrogen come away with difficulty.

When water rises as springs or wells in the bottom of the fish ponds themselves, it is still more difficult of correction, and quite impossible unless the head is strong enough to lift the water above the level of the surface of the pond, and so permit the adoption of the above measures.

It is a fact of significance and importance, to be considered from the standpoint of fish culture, that spring waters may vary considerably from time to time in the amount of dissolved air they contain. An instance of this, recently observed, concerned the oxygen alone, a marked deficiency being followed after several days and subsequent to a heavy rain, by a fairly abundant supply. It is inferred that nitrogen variations may likewise occur, and presumably changes in the solids in solution. Weather and seasonal conditions probably are contributing causes of this variability, but not many observations have been made and little is known beyond the fact, which makes it necessary not to place entire reliance on one examination of a given water.

In three instances of gas disease at government fish-cultural stations the excess of air has been actually determined by analysis. In others similar symptoms make a presumption of a similar cause. Meager information of other cases of disease or mortality among fishes with gas symptoms indicate with more or less probability the presence of supersaturation. A spring at an abandoned private trout cultural establishment in Vermont was found to be constantly giving up large bubbles of air (Table I, page 372, sample 5). Trout culture was not successful in this water, and the former superintendent gave a history of bulging eyes. Analyses were not made, but it seems extremely probable that this water was supersaturated.

In 1902, at the exhibit of the United States Fish Commission at the Charleston Exposition, a sudden and severe loss occurred among the marine fishes of the aquaria. The water precipitated quantities of gas, and the fishes were described as showing external bubbles and blisters of gas. The water supply was obtained by pumps with a long suction. The presumption is strong that the mortality was from excess of air, and that its sudden disappearance was caused by a change in the suction pipe, which, though unwittingly, corrected undetected leaks. The trouble was not explainable on other grounds than these.

The selection of water supplies for fish cultural or similar purposes should include a careful scrutiny of their quality with respect to dissolved air. There are the two opposite faults to be guarded against. When either is extreme in degree, its recognition will not be difficult. But it is probable that cases will occur, and have occurred, where either fault is but slight, and causes no heavy losses or marked symptoms on its own account, while it at the same time is responsible for a gradual and insidious lowering of condition among the fishes which makes them susceptible to the sudden and rapid epidemics of bacterial or protozoan infection, or to the less acute attacks of higher parasites. In such cases the certain recognition of a slight excess of nitrogen, with ordinary methods of gas analysis, may require the average of a number of determinations. The constant ebullition of gas in bubbles of moderate or large size from the water sources is sufficient to cause suspicion of a nitrogen excess, but the absence of such bubbles is by no means reassuring, for supersaturation may occur in the depths of the spring without any of the undissolved residual gas revealing itself at the surface. As for the oxygen, it is not known just what content short of saturation completely supplies all the needs of fishes, but since their natural abodes, and particularly trout streams, closely approach saturation (Hofer 1904, pp. 157 et seq.), it is well to lay stress upon the desirability of maintaining a high oxygenation in fish cultural waters. For trout, and particularly the brook trout, this is imperative. It is probable that most spring waters are not highly oxygenated. Usually they take up incidentally, in the conduits or at delivery pipes, more or less oxygen before they are actually used as a fish-cultural supply, and sometimes means of aeration are specifically provided. So important are these that it seems not too much to say that devices for this express purpose should be provided in all cases where spring or well waters are used for salmonoids, unless repeated quantitative determinations made at different seasons show that the water can not be improved.

Table I.—*Showing composition of gas delivered from the bottoms of ponds, springs, or wells.*

[All gas determinations by M. C. Marsh save where otherwise stated.]

Source of sample.	Date.	Percentage of—			Remarks.
		Carbon dioxid.	Nitrogen.	Oxygen.	
1. Spring at Fishery, Tenn	May, 1903	0.8	82.5	16.7	Continual evolution of gas in large amount.
2. Artesian Well, Nashua, N. H.	Sept., 1903	.4	87.8	11.8	Discontinuous evolution of gas in small amount.
3. Fish cultural pond, Nashua, N. H.	Sept., 1903	.8	82.8	16.4	
4. Reservoir pond, Nashua, N. H.	Apr., 1904	1.4	96.3	2.3	
5. Spring in Vermont	Sept., 1903	Trace.	87.4	12.6	

Nos. 1, 2, and 5 were determined by the Bureau of Chemistry.

TABLE II.—*Showing composition of gas in lesions of the gas disease.*

Source of sample.	Percentage of— Carbon dioxid.	Nitrogen.	Oxygen.	Size of gas sample in c.c.	Remarks.
Hearts of tomcod, sculpins, and hake at Woods Hole.	0	97.44	2.56	3.9	Possibly slight contamination with atmospheric ai in taking sample.
Body of lobster, Woods Hole...	0	94.2	5.8	3.4	Do.
Fin blebs of tomcod, flat-fish and hake.	0	92.1	7.9	7.6	
Eyes of exophthalmic scup, Woods Hole.[a]	0	80.4	19.0	[b]10	
Sacs of rainbow trout fry, White Sulphur Springs, W. Va.	0	92.3	7.7	2.6	Fry preserved in formalin.

[a] Analysis by Dr. M. X. Sullivan.

[b] About.

NOTE.—None of the specimens from which gas samples were taken had been dead over thirty hours, and most of them a much shorter time. During this period they were in water at 10.5° C. There was no sign of putrefaction. The sample from the eyes of scup was taken immediately after the fish were killed.

TABLE III.—*Showing nitrogen and oxygen content (in cubic centimeters per liter, reduced to 0° C. and 760 mm., dry) of Woods Hole sea water under various conditions.*

Source.	Date.	Temperature of water, centigrade.	Actual content c.c. per liter.		Normal content of sea water when saturated with air at the given temperature and prevailing pressure, in c.c. per liter.		Excess + or deficit — c.c. per liter.		Remarks.
					Dittmar.				
			Nitrogen.	Oxygen.	Nitrogen.	Oxygen.	Nitrogen.	Oxygen.	
Harbor under wharf.	1904. May 9	10.0	12.6	6.0	12.37	6.39	+0.23	—0.39	Harmless to fishes.
	No air entering the suction.								
Hatchery tap......	May 8	11.5	12.9	5.9	12.19	6.29	+0.71	—0.39	Harmless to fishes.
Aquarium tap.....	May 9	11.0	12.7	5.8	12.16	6.28	+0.54	—0.48	Harmless to fishes.
	With much air entering suction.								
Aquarium tap.....	May 10	9.75	17.73	8.16	12.54	6.49	+5.19	+1.67	Rapidly fatal to fishes.
Aquarium tap.....	May 10	9.75	18.23	8.34	12.55	6.49	+5.68	+1.85	Rapidly fatal to fishes.
Aquarium tap.....	May 10	10.0	18.79	8.54	12.48	6.45	+6.31	+2.09	Rapidly fatal to fishes.
Aquarium tap.....	May 11	10.5	18.01	8.06	12.37	6.38	+5.64	+1.68	Rapidly fatal to fishes.
Aquarium tap.....	May 11	10.5	18.79	8.41	12.37	6.38	+6.42	+2.03	Rapidly fatal to fishes.
	With a lesser amount of air entering suction.								
Aquarium tap.....	May 12	11.0	15.66	7.06	12.34	6.37	+3.32	+0.69	Less rapidly fatal.
Aquarium tap.....	1903. Sept. 18	20.8	12.5	4.9	10.28	5.24	+2.2	—0.34	Transported sample. U. S. Bureau of Chemistry.

TABLE IV.—*Showing nitrogen and oxygen content (in cubic centimeters per liter, reduced to 0° C. and 760 mm., dry) of various (fresh) waters at and near fisheries station, Nashua, N. H.*

Source of sample.	Date.	Temperature of water, centigrade.	Actual content c. c. per liter.		Normal content when saturated with air at given temperature, and prevailing pressure c. c. per liter.		Excess + or deficit — c. c. per liter.	
			Nitrogen.	Oxygen.	Pettersson and Sondén.	Winkler.	N.	O.
					N.	O.		
	1904.							
Hatchery well No. 1	Apr. 26	8.0	17.5	3.3	16.00	8.26	+1.5	—4.96
Same, second determination	Apr. 28	8.0	18.1	3.4	16.00	8.26	+2.2	—4.86
Same, deaerated drop by drop	Apr. 29	9.5	15.0	7.4	15.35	7.89	—0.35	—0.49
Hatchery well No. 5	May 3	8.0	17.8	2.9	16.14	8.33	+1.66	—5.43
Hatchery well No. 11	Apr. 30	8.0	18.6	1.6	15.72	8.11	+2.88	—6.51
Well in rearing pond No. 3	Apr. 27	8.0	17.5	3.1	16.00	8.26	+1.6	—5.16
Well in rearing pond No. 16	Apr. 28	8.0	17.9	3.8	16.00	8.26	+2.0	—4.46
Reservoir pond water	Apr. 27	8.75	17.2	5.0	15.77	8.13	+1.43	—3.13
Same, through deaerating box	....do ...	8.75	16.4	6.7	15.73	8.11	+0.67	—1.41
Creek water, total station flow, aerated and deaerated by natural flow	Apr. 30	8.5	16.0	6.8	15.61	8.05	+0.39	—1.25
Largest Pennichuck well, source of Nashua city supply	May 2	11.5	17.6	2.1	14.94	7.65	+2.66	—5.55
Smaller Pennichuck well	....do ...	11.5	18.3	2.2	14.94	7.65	+3.36	—5.45
Pennichuck water from service tap at hatchery	Apr. 29	7.0	15.4	6.6	16.24	8.40	—0.84	—1.80
Colerain Brook, a well-aerated natural stream	Apr. 30	12.0	14.5	7.0	14.45	7.39	—0.05	—0.39
Rain water freshly caught	Apr. 29	11.0	14.8	6.6	14.87	7.61	—0.07	—1.01

NOTES.—Presumably normal waters show, according to Tables III and IV, slight nominal excesses or deficiencies of nitrogen, and always a deficiency of oxygen. These discrepancies represent limits of accuracy of apparatus and methods as used in the field, and the personal equation. Moreover, saturation data vary within rather wide limits.

The figures for the dissolved CO_2 are not included in the tables, as having no particular relation to the present subject. They are considerably higher for fresh water containing a nitrogen excess than for normal water, the former averaging 5.3 c. c. per liter with extremes of 3.6 and 7.4, the latter 1.8 with extremes of 1.6 and 2.1. These figures include the semibound carbonate.

SUMMARY.

1. Fishes and some other organisms show gas symptoms of considerable variety and of a pathologic nature. Many of these are due to one cause and may be grouped together as a pathologic unity, the gas disease. An exophthalmia, or pop-eye, is one of the chief lesions.

2. Bacteria are not in any way concerned in the gas disease here considered, but may cause similar lesions.

3. The immediate cause of death in the gas disease is usually asphyxiation from gas embolism in the gill filaments, or heart, or both.

4. This embolic gas is due to an excess of dissolved air in the blood, which may be immediately caused by a rapid reduction of pressure, or by an excess of dissolved gas in the water, or by a combination of both.

5. The form of the disease caused by the reduction of pressure alone occurs only experimentally, or possibly in the case of some deep-sea fishes brought to the surface.

6. The form of the disease caused by an excess of dissolved air alone is the normal one. Nitrogen excess is more important than oxygen excess and can singly cause the disease process.

7. An excess of about 2 c. c. of nitrogen per liter of water is sufficient to cause symptoms. An excess of about 6 c. c. per liter, accompanied by an excess of about 2 c. c. of oxygen, experimentally produced, has been observed in sea water, and kills most adult fishes in a few hours.

8. A certain increase of pressure will prevent the gas disease where otherwise it would occur, and may cure affected fishes. It acts by changing the saturation point so that the excess of air no longer exists.

9. The supersaturated water may be corrected and become harmless by deaeration. This occurs spontaneously upon standing or may be more quickly accomplished by subdividing the water mechanically to offer a great area of exposure to the atmosphere. This process corrects either an excess or a deficiency of air. The water of shallow brooks arising in supersaturated springs or wells is soon corrected by the natural flow.

BIBLIOGRAPHY.

1879. Kidder, J. H.—The Animal Heat of Fishes. Nature, London, Vol. XXI, p. 156.

1884. Dittmar, W.—Composition of Ocean Water. Report on the Scientific Results of the Exploring Voyage of H. M. S. *Challenger*, Vol. I, Part I.

Regnard, P.—Note sur les conditions de la vie dans les profondeurs de la mer. Comptes Rendus Société de Biologie, T. XXXVI, 1884, pp. 164, 187, 310, 394.

1895. Foster, M.—A Text Book of Physiology. Part II.

1896. Comey, A. M.—A Dictionary of Chemical Solubilities.

Fredericq, L.—Sur la tension des gaz du sang arteriel et la théorie des échanges gazeux de la respiration pulmonaire. Archives de Biologie, T. XIV, pp. 105–118.

1898. Gorham, F. P.—Some Physiological Effects of Reduced Pressure on Fish. Journal of the Boston Society of Medical Sciences, Vol. III, p. 250.

Haldane, J. S.—Secretion and Absorption of Gas in the Swimming Bladder. Science Progress, Vol. VII, p. 120.

Lewis, Frederic T.—The Physiological Effect of Compressed Air. Boston Medical and Surgical Journal, Oct. 6, p. 338.

1899. Barbour, E. H.—Wells and Windmills in Nebraska. U. S. Geological Survey, Water-Supply and Irrigation Paper No. 29.

Gorham, F. P.—The Gas Bubble Disease in Fishes and its Cause. Bull. U. S. Fish Commission, Vol. XIX, pp. 33–37.

1900. Wisner, J. N.—Report on Battery Station, Havre de Grace, Md., Rept. U. S. Fish Commission, p. 50.

1901. Hammarsten, Olof.—A Text Book of Physiological Chemistry.

1902. Hempel, Walther.—Methods of Gas Analysis. Translation by L. M. Dennis.

1903. Hill, Leonard, and MacLeod, J. J. R.—Caisson Illness and Diver's Palsy. An Experimental Study. Journal of Hygiene, Vol. III, pp. 401–445.

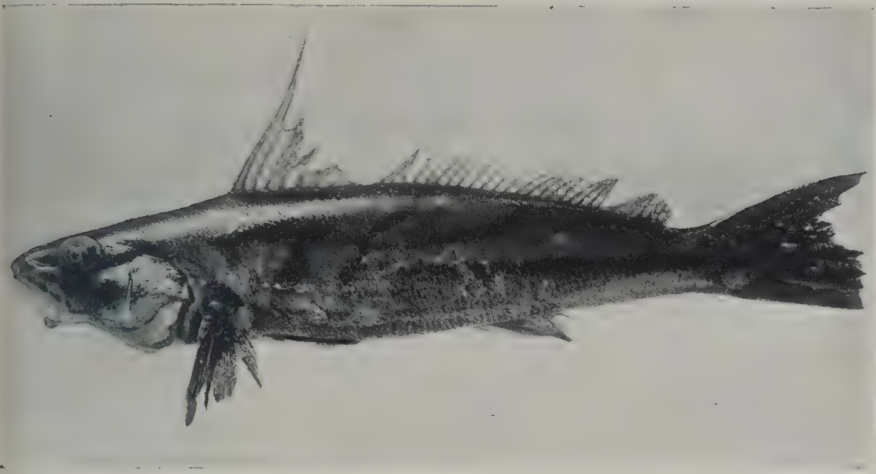
Marsh, M. C.—A Fatality among Fishes in Water containing an Excess of Dissolved Air. Transactions American Fisheries Society, p. 192.

Wolf, L. P.—Experimentelle Studien über Luftembolie, Virchow's Archiv., Bd. 174, H. III, s. 454.

1904. Greene, James S.—The Presence of Air in the Veins as a Cause of Death. American Journal of Medical Science, December, p. 1058.

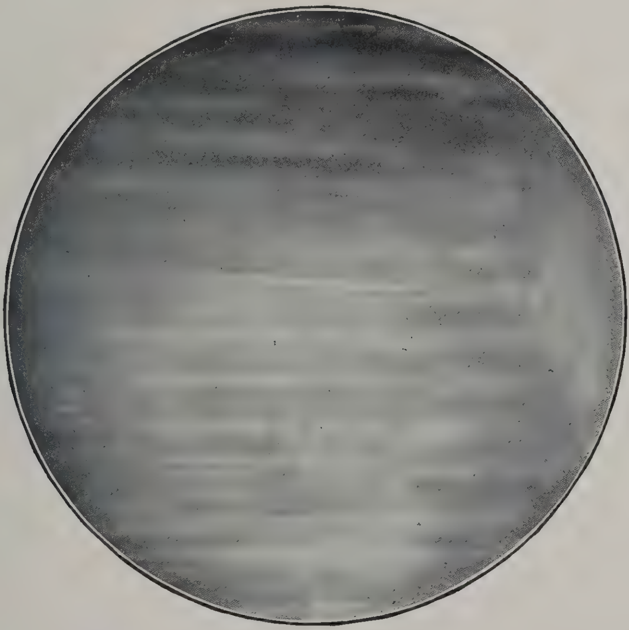
Hofer, Bruno.—Handbuch der Fischkrankheiten. München.

Marsh, M. C.—Exophthalmia, or Pop-eye, in Fishes. American Fish Culturist, August, p. 5.



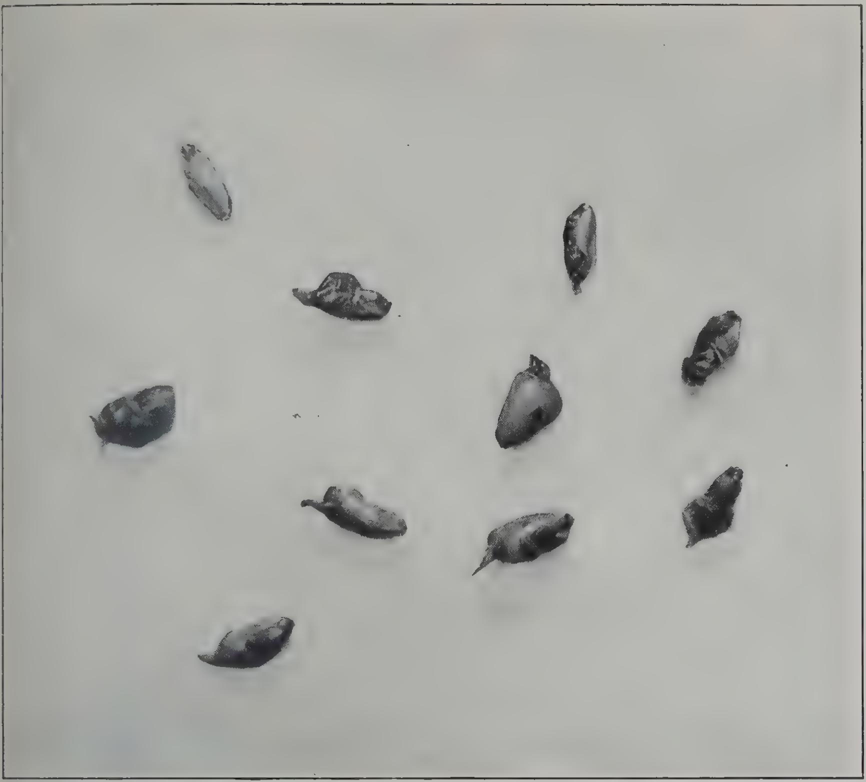
1. A DEAD KINGFISH (MENTICIRRHUS) WITH EXTERNAL LESIONS.

The pop-eye is marked, and vesicles of gas appear in all the fins and in the skin behind the pectoral. The illustration gives a quite inadequate idea of the striking appearance of the specimen as seen alive in the water. (Photograph by J. G. Hubbard.)



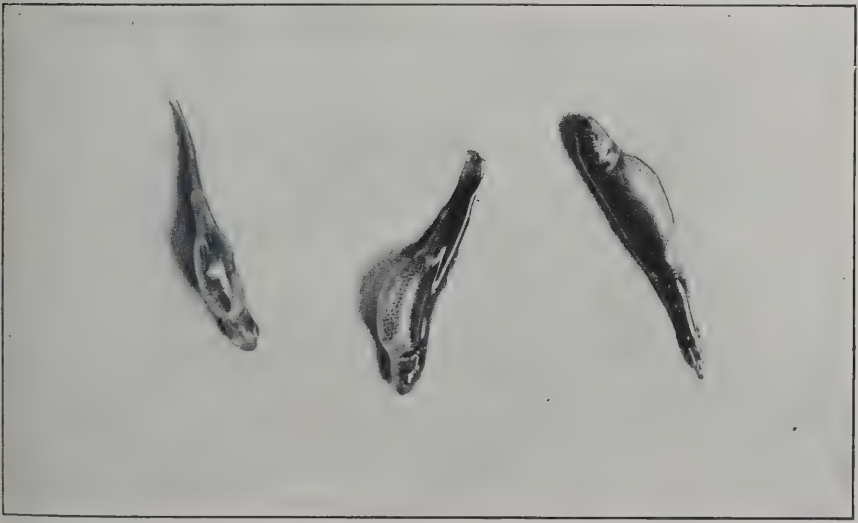
2. THE GILL FILAMENT OF A FISH UNDER A LOW MAGNIFICATION, SHOWING GAS EMBOLI IN THE LUMINA.

(Photograph by J. G. Hubbard.)



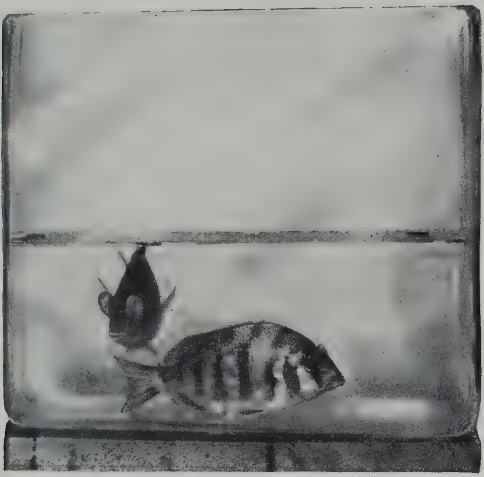
1. YOUNG PUFFERS (SPHEROIDES) WITH GAS DISEASE INFLATION.

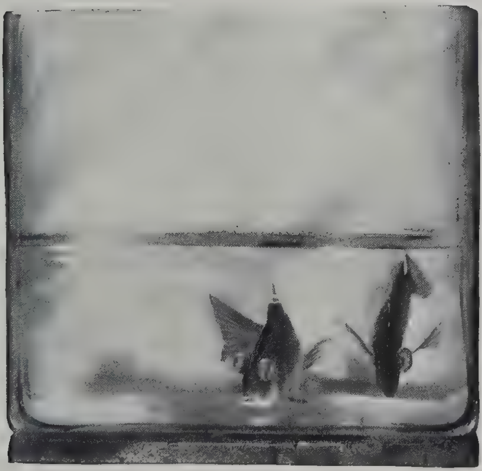
(Photograph by F. P. Gorham.)



2. RAINBOW TROUT (SALMO IRIDEUS) FRY ABOUT 6 WEEKS OLD, SHOWING DISTENTION OF THE ABDOMEN WITH GAS.

(Photograph by T. Surber, White Sulphur Springs, W. Va.)





LIVING SCUP WITH POP-EYE ACQUIRED DURING 5 DAYS.

The eyeball has been forced only slightly from its seat, while the conjunctiva has been greatly inflated by the gas. (Photographs by J. G. Hubbard, Woods Hole, Mass.)

SYNTHETIC CULTURE MEDIA AND THE BIOCHEMISTRY OF BACTERIAL PIGMENTS.

BY M. X. SULLIVAN.

Journal of Medical Research, Vol. XIV, No. 1, (New Series, Vol. IX, No. 1,) pp. 109-160, November, 1905.

A Thesis submitted to the Faculty of Brown University for the degree of Doctor of Philosophy.

SYNTHETIC CULTURE MEDIA AND THE BIOCHEMISTRY OF BACTERIAL PIGMENTS

(*THESIS SUBMITTED TO THE FACULTY OF BROWN UNIVERSITY*)

BY

MICHAEL XAVIER SULLIVAN

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SYNTHETIC CULTURE MEDIA AND THE BIOCHEMISTRY OF BACTERIAL PIGMENTS.*

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INTRODUCTION.

Bacteria, the smallest and at the same time the most important of the microörganisms, are divided into two groups, a lower and simpler and a higher and better developed. The members of the higher group take little part in the great problems of fermentation, decomposition, and disease, and consequently have been less extensively studied than the simpler forms. The members of the lower group, which at present command most of the attention of the bacteriologist, are more numerous and consist of minute unicellular masses of protoplasm.

For a long time after their discovery by Leuwenhoek in 1675, these simple organisms were regarded as animals. Even as late as 1848 Nägeli ('48), who gave to bacteria their present name of schizomycetes, declared that it was impossible to say whether they were animals or plants. At the present time, however, bacteria, though resembling the flagellata as regards their motility, are considered as belonging to the plant kingdom and are classified as schizomycetes or fission fungi. Though classified as fungi, mainly on account of the lack of chlorophyll, morphologically and developmentally the bacteria are more closely related to blue-green fission algæ or cyanophyceæ, from which they are in many cases distinguished only through their peculiar movements and lack of phycochrome.

Indeed, to make a clear distinction between the lowest protophytes is difficult. It has long been asserted that a sharp line separates the algæ, which contain chlorophyll, and the fungi, which are lacking in this substance. With the

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* Received for publication Aug. 27, 1905.

aid of the chlorophyll, the algæ, like the higher plants, can build up their protoplasm from such simple compounds as carbon dioxide, ammonia, and salts which contain nitrogen. The fungi, on the other hand, having no chlorophyll, were supposed to be absolutely dependent upon pre-formed organic matter. This distinction no longer holds between the cells containing chlorophyll and those devoid of it. We now know that among the phanerogams there are many colorless plants, and that among the bacteria, on the other hand, there are certain pigmented forms which approach closely to some of the lower Cyanophyceæ. Thus Bacterium viride, B. virens (van Tieghem), and B. chlorinum Englemann (Chester, '01), are tinted green by a substance which, with some doubts, may be regarded as chlorophyll.

Whether bacteria contain chlorophyll or not, it is nevertheless true that many of them can derive their nourishment from extremely simple compounds. Bolton ('86) found, for instance, that B. aquatelis Flügge and B. erythrosporus could live and multiply in doubly distilled water. Winogradsky ('90 and '91), Jordan and Richards ('90) have shown, in addition, that the nitrifying bacteria thrive best on a solution of such simple compounds as ammonium chloride, sodium carbonate, sodium phosphate, and potassium sulphate, without any traceable amount of organic matter. Pasteur, one of the first to conduct experiments upon the biology of the lower organisms, showed, moreover, that yeasts (and moulds also) could, like the higher plants, secure their nitrogen from ammonia salts and even from nitrates, and could, like chlorophyll-containing plants, build up the protoplasm of their bodies from simple compounds. More recently, also, Uschinsky ('93 and '97) has shown that a complete synthesis of albuminous matter can be made by chlorophyll — free bacteria growing upon a solution of simple chemicals, the most complex of which was asparagin, the amide of amido-succinic acid. Lastly, Winogradsky ('93) has shown that the root tubercle bacteria, as B. radicicola, are able to assimilate the atmospheric nitrogen and to obtain their carbon from carbon dioxide. Indeed, plants are dependent upon these bacteria to convert ammonia into nitrous and nitric acid, from which forms plants can secure their nitrogen.

Though the relation of bacteria to other plants has been recognized for some time, and despite the fact that it has been shown that certain varieties can thrive on simple compounds, few experiments have been made to test their activity on simple culture media. On the contrary, with few exceptions, a complex albuminous medium has been in use since Müller, in 1876, made his first attempt at the systematic study of bacteria. At the present time the standard medium is a solution of meat infusion and peptone, with the addition of agar or gelatin when a solid medium is wanted.

By the use of meat infusion and peptone solidified by the addition of agar or gelatin, great advance has been made in bacteriology. The highest degree of accuracy, however, cannot be reached by their use, owing to the fact that meat infusion and peptone vary widely in their composition and in their nutritive value. The chemical composition of the media now in standard use is not even qualitatively known, so that it has been impossible to make a satisfactory chemical study of the life activities of the bacteria, such as the production of pigment, toxins, or metabolic products.

Believing that bacteria could derive their nutriment from simple media, the composition of which we may know, qualitatively and quantitatively, and that upon simple media the problems of color production and metabolic products, hitherto guessed at for the most part, could be studied, I have undertaken:

1. To replace wholly or in part the present standard culture media by simpler known compounds.

2. To determine the conditions under which bacteria develop their pigments.

3. Knowing the composition of the food-stuff, to find out what are the metabolic products.

The bacteria used in all the investigations were secured from this laboratory, from the Bacteriological Laboratory of the Harvard Medical School, from the Biological Laboratory of the Massachusetts Institute of Technology, and from Kral's Laboratory in Prague.

In the synthetic work, the ordinary saprophytic and pathogenic bacteria were employed. In the study of pigment, the results are based upon an investigation of the following bacteria described in Migula's "System der Bakterien," and Flügge's "Mikro-organismen."

Name.	Described.		Color.
		Page.	
B. pyocyaneus Gessard	Migula,	884.	Blue.
B. fluorescens liquifaciens	"	913, 926.	Blue-green.
B. fluorescens non-liquifaciens	"	913, 926.	" "
B. fluorescens immobile	Flügge, Vol. II.,	294.	" "
B. cyaneo-fluorescens	Migula,	906.	Blue-black.
B. prodigiosus	"	419.	Red.
B. ruber balticus	Flügge, Vol. II.,	303.	"
B. rosaceus metalloides	Migula,	938.	"
B. havaniensis	"	725.	"
M. roseus	"	176.	Pink.
M. mycoides roseus	Flügge,	205.	"
M. agilis	Migula,	275.	Brick red.
B. violaceus	"	939.	Violet.
B. janthinus	"	941.	"
M. citreus	"	147.	Yellow.
S. pyogenes citreus	"	147.	"
M. tetragenus versatilis	Sternberg,	613.	"
S. aurantiaca	Migula,	238.	Yellow-orange.
M. cereus flavus	"	126.	" "

Part I.

SYNTHETIC CULTURE MEDIA.

1. *Elimination of the Meat Infusion.*

An analysis of the standard meat infusion, sterilized and filtered, shows but small amounts of albumoses and peptone together with sodium, potassium, and traces of magnesium, calcium, and iron as bases, and hydrochloric, sulphuric, phosphoric, and organic acids. Considering the small percentage of organic matter present in the meat infusion, it seemed possible to replace the latter altogether by Witte's peptone and salts.

Accordingly, leaving out the meat infusion, a medium containing the bases and acids found by analysis, together with Witte's peptone, was made up in distilled water as follows:

Witte's peptone	1.5	per cent.
$NaCl$	0.3	" "
K_2HPO_4	0.15	" "
$MgSO_4$	0.05	" "
$Fe_2(SO_4)_3$	trace.	
$CaCl_2$	"	
Ammonium lactate	0.05	" "
Glycerin	2.00	" "
Agar	1.00	" "

Upon this medium over seventy kinds of bacteria developed, including putrefactive, phosphorescent, chromogenic, and pathogenic varieties. An attempt was then made to eliminate one after another of the salts and to reduce the medium to the lowest terms capable of giving a good growth of bacteria. The omission of $CaCl_2$ and $Fe_2(SO_4)_3$ did not materially diminish the number of bacteria which grew upon the medium, nor was the growth less abundant.* Again, leaving out NaCl we have medium B.

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* Since the general aim of this work was to find out what combinations of salts would be best adapted for pigment production, no great attempt was made to learn how many bacteria would grow upon these synthetic culture media, nor how well they would grow upon such media as compared with the growth upon standard media.

(B.)	Witte's peptone	1.00	per	cent.
	$MgSO_4$	0.05	"	"
	K_2HPO_4	0.1	"	"
	Ammonium lactate	0.05	"	"
	Glycerin	2.00	"	"
	Agar	1.00	"	"

We had still a very abundant growth of at least fifty varieties, including twenty-five pigment producers, reds, yellows, violets, blue-greens, etc., as well as B. typhosus, B. coli, B. pneumoniæ, B. anthracis. The pathogenic grew somewhat more slowly than an ordinary media, but the water bacteria and pigment producers seemed to thrive as well if not better than on the standard culture media. Reducing the salts still further, the number of bacteria that would grow with any degree of rapidity decreased, though, upon C.

(C.)	Peptone	2.00	per	cent.
	NaCl	1.00	"	"
	Agar	2.00	"	"

Twenty-five varieties grew, among them Microspira comma, B. anthracis, B. pneumoniæ, photogenic, and chromogenic bacteria. The growth, however, was slow, and the pigment production was very slight. Even upon a one per cent solution of peptone some varieties grew, and the chromogenic bacteria still gave a slight formation of pigment.

2. *Elimination of the Peptone.*

Since the peptone varies in nutrient value and contains impurities such as phosphates, sulphates, and chloride of potassium, sodium, and magnesium, we next endeavored to replace it by similar chemical compounds whose composition is known definitely. As the nitrogenous basis of these media we used asparagin or ammonium salts. Asparagin or amidosuccinic-acid-amide, having a formula $COOH, CHNH_2, CH_2, CoNH_2$, has been ascertained by Neelsen, Nägaeli, and Hueppe (Hueppe, '99) to be one of the best substances for the nutrition of bacteria. According to Hueppe, indeed, its nutritive value is such that even some warm-blooded animals, such as cattle, can make use of it to a certain extent as a poor substitute for proteid.* Replacing the proteid by asparagin we make solution D.

(D.)	Asparagin	1.0	per cent.
	Dextrose	0.5	“ “
	$MgSO_4$	0.03	“ “
	K_2HPO_4	0.1	“ “
	KNO_3	0.1	“ “
	NaCl	0.1	“ “

Upon D upwards of thirty varieties developed quickly. Among them were water bacteria, including B. coli, certain chromogenic bacteria, and pathogenic forms, including B. typhosus, B. anthracis, and B. diphtheriæ. In the last two cases the growth was slight and slow. On this medium the sugar, though yielding its carbon, hydrogen, and oxygen readily, was somewhat unfavorable, since it gave rise to acids which changed the original slight alkalinity to a degree of acidity which was prohibitive not only to a good formation of pigment, but also to growth.

Accordingly, replacing the dextrose by glycerin, we have medium E.

(E.)	Asparagin	1.5	per cent.
	K_2HPO_4	0.1	“ “
	$MgSO_4$	0.02	“ “
	Ammonium lactate	0.08	“ “
	NaCl	0.1	“ “
	KNO_3	0.03	“ “
	Glycerin	1.00	“ “

On this medium about forty varieties grew, including twenty chromogenic forms such as S. pyogenes citreus, B. pyocyaneus, B. fluorescens liquifaciens, B. violaceus, and B. prodigiosus, B. ruber balticus, B. rosaceus metalloides; and pathogenic forms, such as B. pneumoniæ, B. typhosus, and to a slight extent B. diphtheriæ. On E the red and violet pigment producers quickly formed their pigment, while the yellow pigment producers, as S. pyogenes citreus, M. aurantiaca, developed slowly and showed pigment only after the lapse of more than twenty (20) days. If the lactate be increased to one-half of one per cent or over, however, no pigment is produced, though the growth is good. Likewise, ammonium tartrate (0.5 per cent) or ammonium malate (0.5 per cent) prove unfavorable to pigment production. Concerning the pigment production, however, we shall have more to say in the second part of this paper.

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* See Mauthuer ('93) and Politis ('93).

In a solution very similar to E, but more unfavorable to pigment production because of the large percentage of ammonium lactate, Uschinsky ('93 and '97) found that pathogenic bacteria, as cholera, tetanus, diphtheria, could grow as well as on bouillon, and in the case of B. diphtheriæ could develop toxin. Furthermore, he found that when the culture was filtered through porcelain it gave the xanthroproteic and Millon's tests for albumens. That bacteria can synthesize from a simple compound to more complex molecules which give the test for proteids, I have found to be the case with B. fluorescens liquifaciens grown on E, and with others grown on still simpler solutions.

As we decrease the number of salts in the medium, the growth of the bacteria is somewhat diminished, though upon F,

(F.) Water.

Asparagin	1.00	per cent.
K_2HPO_4	0.1	" "
$MgSO_4$	0.02	" "
Glycerin	2.00	" "

the chromogenic bacteria for the most part developed rapidly with good production of color. Thus, upon this medium the pigment-producing bacteria developed as follows:

(A.) Those producing pink or red colors, as B. prodigiosus, B. ruber balticus, B. rosaceus metalloides, B. rhodochrome, B. havaniensis. Of these, the first three gave the pigment in from twenty-four to sixty hours; B. rhodochrome more slowly and far less abundantly. B. havaniensis, on the other hand, produced its pigment only after the lapse of several weeks, and then merely as slight sediment.

(B.) Blue-green and fluorescent greens, as B. pyocyaneus (I.) producing blue only; B. pyocyaneus (II.) producing the pyocyanin soluble in chloroform, and in addition a blue-green fluorescent pigment insoluble in chloroform; B. pyocyaneus (III.) originally producing both pyocyanin and fluorescent pigment, but now producing the fluorescent pigment only; B. fluorescens liquifaciens.

(C.) Violet pigments, B. violaceus and B. janthinus. These bacilli produced these pigments well.

(D.) Yellow pigments, as S. pyogenes citreus, M. citreus, B. fuscus, and S. aurantiaca. In the case of the yellow pigment producers, the colors appear only after weeks and even months of growth, and then only as a slight sediment. Upon this medium F the following, though growing more or less, did not form any pigment: B. miniaceus, red; S. rosea, rose red; B. ruber (Plymouth), pale red; M. cinnabareus, pale pink; M. agilis, brick to bright red; M. mycoïdes roseus, rose red; B. cyaneo-fluorescens, producing a blue-green and a black; S. lutea; M. tetragenus versatilis; M. cereus flavus, yellow forms. Upon medium G,

(G.)	Asparagin	1.00	per cent.
	NaCl	1.00	“ “
	$MgSO_4$	0.3	“ “
	Agar	1.00	“ “

varieties such as Microspira comma and B. pneumoniæ showed good growth, but most of the chromogenic bacteria were colorless. B. pyocyaneus, however, produced abundant pigment. Even upon medium H,

(H.)	Glucose	2.00	per cent.
	Na_3PO_4	0.5	“ “
	K_2HPO_4	0.5	“ “
	Agar	1.00	“ “

several water bacteria were able to develop. In this case, probably, the water bacteria derive their nourishment from the nitrogenous and other salts which exist in agar as impurities. It has been ascertained, moreover, that water bacteria can develop on agar and asparagin alone, in which simple medium B. coli will not grow.

3. *Colloidal Silicic Acid as a Substitute for Agar or Gelatin.*

As bases for solid culture media, agar and gelatin are of great use. Agar, however, undoubtedly contains many impurities, as sea salts and diatoms. Gelatin likewise is variable in composition. If an organic colloid of known purity could be adapted to the needs of bacteria, it would be of great use in the study of pigment production, in the reduction of nitrates, and in all work requiring greater exactness than is possible on the present colloidal bases — agar and gelatin. Accordingly, an endeavor was made to replace the colloids now in use by the inorganic silicic acid colloid. Kühne ('90), Winogradsky ('90), and Sileskin ('91) have already done some work on colloidal silicic acid, and found it of great use where a medium free from organic matter was wanted. They made the colloid by neutralizing water glass or sodium silicate by hydrochloric acid and then dialyzing off the excess of acid. This method, however, is too troublesome for ordinary use and does not give a firm colloid. The following method is much better, since it is simpler and gives a firmer colloid. To the solution containing the various salts to be used, about four per cent of sodium silicate was added. This percentage gives the best colloid. Then three and one-half per cent glycerin, which is of great use in preventing silicic acid colloid from throwing out its water, was mixed with the solution. After the mixture was sterilized and cooled there was added, drop by drop, with frequent stirring, enough of a ten per cent solution of phosphoric acid to render the solution slightly acid to phenolphthalein. In about thirty minutes, at the room temperature, the medium solidifies to a firm gelatinous mass. In the sterilizer the medium is solidified in from five to ten minutes. This silicic acid colloid I have found of use in the study of color production.

4. *Practical Application of Synthetic Media.*

It would seem that some bacteria can thrive on certain media better than others. Thus on medium K,

(K.)	Asparagin	1.00 per cent.	
	Glycerin	3.5 “ “	Slightly acid by evaporation of NH_3 in sterilizing.
	K_2HPO_4	0.1 “ “	
	$(NH_4)_3PO_4$	1.0 “ “	
	$MgSO_4$	0.2 “ “	
	Agar	1.0 “ “	

ordinary water bacteria and B. coli grew well, while B. typhosus showed hardly a trace of growth. This difference in the growth of B. coli and B. typhosus on certain media has been taken advantage of to differentiate between them. Thus Capaldi and Proskauer ('97) have devised a medium in which B. coli grows well and freely produces acid, while the typhoid bacillus hardly grows at all and produces no change in the reaction. Its composition is:

Asparagin	0.2 per cent.
Mannite	0.2 “ “
NaCl	0.02 “ “
$MgSO_4$	0.1 “ “
$CaCl_2$	0.02 “ “
K_2HPO_4	0.2 “ “

5. *Ferments.*

In order to find out whether bacteria would secrete pigments on synthetic media, I grew B. prodigiosus and B. pyocyaneus on a medium consisting of:

(J.)	Asparagin	1.00 per cent.
	$MgSO_4$	0.02 “ “
	$CaCl_2$	trace
	K_2HPO_4	0.1 “ “
	NaCl	0.1 “ “
	KNO_3	0.02 “ “
	Gelatin	10.00 “ “

The gelatin was liquefied by both species. Further, I have found that B. prodigiosus growing on a simple medium like J, neutralized and sterilized by a heat of 55° C. for five minutes, speedily caused a solid coagulation of milk. Fermi ('90 and '92) made an extensive study of the enzymes produced by bacteria, but came to the conclusion that few, if any, bacteria produced enzymes upon non-albuminous media. As regards diastatic and invertin enzymes, I must agree with Fermi's conclusions, but as regards the formation of proteolytic and rennin-like enzymes I must say that they are formed on my synthetic media.

6. *A Synthetic Medium for the Laboratory.*

Though many species of bacteria can work synthetically just as easily as analytically, there are some kinds which are adapted to the one method, others to the other. B. subtilis, for example, shows little power to develop on simple media. On the whole, however, a synthetical medium like the following would be of great help in the laboratory:

(1.)	Asparagin	1.00	per cent.
	$MgSO_4$	0.02	" "
	K_2HPO_4	0.1	" "
	Ammonium lactate	0.05	" "
	NaCl	0.5	" "
	KNO_3	0.02	" "
	Glycerin	1.00	" "
	Agar	2.00	" "

7. *Conclusions.*

These experiments show that bacteria can in a great measure be grown on simple media, and that from these simple combinations the microörganisms can elaborate not only the protoplasm of their bodies but also material which, passed through a porcelain filter, gives the xanthroproteic and Millon's reaction for albumens. Not only can bacteria synthesize from simple to complex, but on simple media they can also carry on other life activities, as the production of pigment, and in some cases, at least, the formation of ferments or enzymes. Since many bacteria, however, show but little power to grow upon synthetic media, it is undoubtedly true that for a general culture medium nothing can replace the nutrient gelatin or nutrient agar. On the other hand, it is possible to develop in an organism the power to grow on simple salts, either by accommodating the medium to the organism or by adapting the organism to the medium.

Only through the study of the vital activity of such simple organisms as bacteria in simple media can we get a definite insight into the finer metabolism of living beings, such as the building of the proteid molecule, the development of pigment and light production, etc. The study of the biochemistry of organisms is fraught with great possibilities. We must recognize with Meldola ('04) that synthesis in the living organism is quite different from synthesis in the laboratory, so that to make much advance in the synthetic chemistry of living vital products, the chemist and the biologist should work hand in hand. While large numbers of definite vital products have been isolated, identified, and synthesized in the laboratory, the course of development of these compounds in the organism can hardly be said to have been traced satisfactorily in any instance. Schützenberger ('88 and '91), Schulze ('78, '80, '97), Halliburton ('95), and Escombe ('98) have done much work in the synthetic chemistry of vital products, but there is still much to be done. If we could successfully trace the chemical evolution in the living organism of one definite compound of known constitution we might discover the lines leading to the fundamental principles of life.

Part II.

THE BIOCHEMISTRY OF BACTERIAL PIGMENTS.

1. *Color in Nature.*

Color in nature may be divided into structural and pigmental. The former are purely physical, depending upon striations on the surface or on a series of their plates, as the iridescence of bristles, cuticle of worms, mother of pearl. The latter are substances of definite chemical composition which by appropriate reagents can be extracted from the colored tissues. They are produced by the activities of animals or plants and react to light in the same way whether they are within the tissues or outside of them. The color of the American Beauty rose, for example, is due to a red pigment present in the cells of the petals, which can be extracted from these cells. The pigments are generally organic compounds, *i.e.*, compounds of carbon and hydrogen. Some of these natural organic pigments have been known for centuries; the ancients obtained their Tyrian purple from various shell fish, such as Murex and Purpura; while in modern times the different preparations of carmine and cochineal, saffron and hematoxylin, or logwood, are familiar examples of pigments produced by animals or plants which are habitually employed as coloring agents. The greater number of plants and animals are, however, too fugitive to be utilized. Furthermore, as it is difficult to obtain the pigment in a pure state, practically nothing is known of their relations and constitution, and indeed scarcely any investigations have been made upon them.

Color in nature has been largely studied as a factor in organic adaptation and the survival of species. Accordingly we find it looked at from the viewpoint of protective coloration, warning colors, mimicry, etc. On the other hand, as Hargitt ('03) points out, the more fundamental problem of the physiological significance of the color or the part it has played in the processes of metabolism of the individual organism has been greatly neglected. Hence in this paper we shall strive to make a more proper interpretation of color in nature by studying its development in bacteria, the simplest of organisms, where sexual selection, warning coloration, protective resemblance, can play no part, and where we may change the conditions of metabolism as we see fit.

2. *Classification of Pigments in Nature.*

Though the chemistry of most of the natural pigments is too imperfectly known to enable us to judge as to their function, it may be well to mention certain categories into which some of them fall. These, according to Newbigen ('98), are:

(A.) Pigments of direct importance, as in respiration.

(B.) Derivatives of such pigments.
(C.) Waste products, or modifications of such.
(D.) Introduced pigments.
(E.) Reserve products, or pigments associated with reserves.

(A.) Pigments of Direct Importance.

Of the pigments of direct physiological importance, we may mention hemoglobin, the red coloring matter of the blood of vertebrates, which possesses the power, owing to the iron it contains, of forming a loose combination with oxygen and so acting as a respiratory pigment. Next we have chlorophyll, a compound of great complexity, which is considered to play a great part in the metabolism of green plants. These plants, when exposed to sunshine can decompose carbonic acid and set free oxygen by virtue of the chlorophyll which they contain. How the chlorophyll does this is hard to say. According to Schunck (Newbigen, '98), however, the chlorophyll carries carbonic acid from the air to the assimilating protoplasm just as hemoglobin carries oxygen.

(B.) Derived Pigments.

The second group of pigments includes those which are formed by the decomposition or breaking down of the pigments of the first group. Though little is known about them, they are regarded as of little importance in producing coloration. Melanin, however, which is supposed to be a derivative of hemoglobin, is the dark pigment of the hair and skin.

(C.) Waste Products.

The third group, or waste pigments, has been studied by F. Gowland Hopkins ('89) in this country, and F. Urech ('93) abroad. The most important of this class of colors are the pigments which produce the beautiful tints in the wings of butterflies. Both Hopkins and Urech have decided that these colors are due to pigments which are normally waste products, or modifications of the waste products of the organism. This utilization of waste products as coloring agents can only occur in cases where colored structures are not intimately connected with the blood system.

(D.) Introduced Pigments.

Introduced pigments are not produced by the organism in which they occur but are obtained from other organisms used as food and are transferred apparently almost unaltered to the tissues. Thus it has been shown by Poulton ('93) that the green pigment of some caterpillars is derived from the green leaves upon which they live.

(E.) Reserve Products.

We next come to reserve pigments — the most widely distributed of all. Most of those classified as reserve belong to a large series known as lipochromes or fat pigments. The lipochromes are pigments varying in color from orange to red which in the dry state give a blue color with concentrated sulphuric or nitric acid, and which are soluble to a greater or less degree in all the solvents of fat. Further, all lipochromes give characteristic reactions: (1) a transient blue violet followed by bright blue when heated with concentrated H_2SO_4; (2) a transient bluish green under the influence of strong nitric acid; (3) an initial green color passing into bluish green by the action of a dilute (2.5 per cent) solution of iodine in a one-half of one per cent solution of potassium iodide. They are divisible into two series according as they do or do not form compounds with the caustic alkalies. Both series occur in animals, but the second only in plants.

The first lipochrome was discovered by Thudichum ('69) in the corpus luteum formed in a graafian vesicle after the discharge of an ovum. The lipochromes are found associated with fat in organisms. Thus from the red flesh of the salmon it is possible to extract an oil containing the pink coloring matter in solution. During the breeding season, when the salmon is fasting, the fat which is so abundant in the muscles is transferred to the ovaries, and with the fat the pigment is also carried, so that the muscles become pale. Since the pigment disappears with the fat, Zopf ('89) describes this kind of pigment as a reserve product. Whether or not the pigment itself is a reserve product, it occurs very frequently in association with reserves. Lipochromes are widely distributed in the animal and vegetable kingdoms — most of the yellow, orange, and red colors being due to them. In plants the best known lipochrome is carotin, which is widely distributed. This carotin, according to Carl Ehring (Newbigen '98), is a cholesterin fat.

3. *Bacterial Pigments.*

Among microörganisms there occur colors of the most varied and brilliant kinds. Red pigments are formed, for example, by many yeasts, by M. cinnabarus, B. prodigiosus, B. indicus ruber, B. rubrum, the red sulphur bacteria, and others; green pigments by B. pyocyaneus and varieties related to it; fluorescent green or green blues by the various varieties of B. fluorescens; blue, by B. cyaneo fluorescens; yellows, by numerous micrococci, sarcinæ, and bacilli. According to Beyerinck ('91), the chromogenic bacteria may be divided into three classes:

(1.) Chromophorus bacteria, in which the pigment is placed in the cell and has a certain biological significance analogous to the chlorophyll of higher plants. In this division belong the green bacteria discovered by Van Tieghen and Engelmann, the red sulphur bacteria which derive their life energy from the decomposition of hydrogen sulphide and, according to Engelmann, give out oxygen.

(2.) Chromoparous or true pigment bacteria, which set free the pigment as useless excretion, either as a colored body or as a leuco-body which becomes colored through the action of atmospheric oxygen. The individuals themselves are colorless and may under certain conditions cease to form pigments. To this class belong B. prodigiosus, B. cyanogenes, B. pyocyaneus, etc.

(3.) Parachrome bacteria, which form the pigment as an excretory product but retain it within their bodies, as B. janthinus and B. violaceus. When the pigment is soluble in water, as those produced by B. pyocyaneus and the fluorescent bacteria, it diffuses through the medium. When the pigment is not soluble it either lies within the cell wall or between the individuals.

(A.) The Previous Work on Pigments.

Considerable work has been done on pigment production, but with few exceptions upon proteid media. Among those who employed non-proteid media in the study of the conditions of pigment production are Laurent ('90), Gessard ('92), Lepierre ('95), Noesske ('97), Jordan ('99), Kuntze ('00), Luckhart ('01), and Hefferan ('04). Jordan has reviewed and improved upon the previous work on bacteria producing pyocyanin and the fluorescent pigment, and has stated conditions under which these pigments are formed. Kuntze has studied the conditions under which B. prodigiosus produces its pigments, while Mary Hefferan carried out a comprehensive and detailed comparative study of bacilli producing red pigment.

As Mary Hefferan has already noted, great variation occurs among different strains of the same bacteria in their ability to produce pigment. In my work, however, I chose for study mainly those varieties that would produce pigment in non-proteid media.

(B.) Classification of Pigment-producing Bacteria into Groups.

As we have already seen in the section on synthetic work, the chromogenic bacteria can produce their color on very simple media. Many of them can form pigment even on such simple medium as a one per cent peptone solution. But since the peptones vary widely in chemical composition and nutritive value, and since Gessard ('92) and Lepierre ('95) proved that at least one pigment, fluorescence, is profoundly affected by the nature of the peptone used, I have carried out my investigations for the most part with the aid of simple solutions of chemical compounds whose molecular composition and arrangement are more accurately known. In treating the pigments I propose to divide them into five groups, namely:

(1). Blue or blue-green pigments produced by such forms as B. pyocyaneus.
(2.) The pigment produced by the varieties of fluorescent bacteria.
(3.) Reds and violets.
(4.) Miscellaneous colors, as B. roseus, M. mycoides roseus, and the black pigment of B. cyaneo fluorescens.
(5.) Yellow and orange.

(1.) Pyocyanin.

Starting with one medium, A,

Asparagin	0.2	per cent.
$MgSO_4$	0.02	" "
K_2HPO_4	0.1	" "
Glycerin	1.00	" "

upon which most of the chromogenic bacteria produced their pigment, the K_2HPO_4 was left out. When the K_2HPO_4 was retained and $MgSO_4$ left out, there was likewise no pigment, but the growth was more abundant than in the previous case.

Taking now the combination:

Asparagin	0.5	per cent.
K_2HPO_4	0.1	" "
Glycerin	1.00	" "

I proceeded to estimate the value of $MgSO_4$ by replacing it by other salts. Since in dealing with such small percentages impurities might easily creep in, I repeated every combination at least three times. In every case I used distilled water and chemically pure salts, either bought as such or recrystallized. In cases of doubt, the salts were recrystallized once. The results were as follows:

(B.)	Asparagin	0.2	per cent.
	K_2HPO_4	0.1	" "
	KNO_3	0.1	" "
	Glycerin	1.00	" "

Upon this medium there was good growth in most cases. B. pyocyaneus formed a slight amount of pigment. Occasionally in the other pigment producers a slight coloration appeared, but as repetition did not verify the production I decided that pyocyanin alone was produced on this solution.

(C.)	Asparagin	0.3	per cent.
	NaCl	0.1	" "
	K_2HPO_4	0.1	" "
	Glycerin	2.0	" "

On this pyocyanin alone was produced.

(D.)	Asparagin	1.00	per cent.
	NaCl	0.02–0.1	" "
	KNO_3	0.02–0.1	" "
	Glycerin	1.00–2.00	" "

On this medium pyocyanin alone was produced. In the case of the other bacteria there was a good growth, and the tubes containing the larger percentages of the ingredients showed the better growth.

(E.)	Asparagin	1.0	per cent.
	K_2HPO_4	0.1	" "
	KBr	0.02–0.1	" "
	Glycerin	1.0 –2.0	" "

In E there was the slightest trace of pyocyanin, but colorless growth in the case of the other pigment producers.

(F.)	Asparagin	2.0	per cent.
	K_2HPO_4	0.1	" "
	KI	0.02–0.1	" "
	Glycerin	2.0	" "

Pyocyanin only was produced.

(G.)	Asparagin	1.00	per cent.
	K_2HPO_4	0.1	" "
	KCL	0.02–0.1	" "
	Glycerin	2.00	" "

On G pyocyanin was readily produced. Several other bacteria showed a trace of pigment.

(H.)	Asparagin	1.00	per cent.
	K_2HPO_4	0.1	“ “
	K_2SO_4 or Na_2SO_4	0.02–0.1	“ “
	Glycerin	2.00	“ “

There was a very good production of pyocyanin. B. fluorescens liquifaciens and non-liquifaciens formed yellowish pigments with very little fluorescence.

(I.)	Asparagin	1.00	per cent.
	K_2HPO_4	0.1	“ “
	Na_2S	0.02	“ “
	Glycerin	2.00	“ “

B. pyocyaneus produced much pyocyanin on I. In the case of the other pigment producers there was a faint shade of color.

It would seem from these experiments that magnesium may be replaced by other salts, as sodium or potassium. Suspecting, however, that the glycerin used was sufficiently impure to furnish the slight trace of magnesium salts that might be necessary, it was decided to leave the glycerin out of the solution. Sugars could not be substituted as they were found to be still more impure.

Both the sugars and glycerin are of great help to the bacteria in producing their pigment on account of the carbon they contain. Since they tend, however, to have inorganic impurities they were both dispensed with. It was then found that the bacteria could produce pigment upon

(K.)	Asparagin	1.00	per cent.
	$MgSO_4$	0.2	“ “
	K_2HPO_4	0.1	“ “

Upon this medium K all varieties of B. pyocyaneus produced their pigments, both pyocyanin and the blue-green to greenish-yellow fluorescence, all varieties of fluorescent pigments, including that produced by cyaneo-fluorescens. Using this medium as a basis, I substituted for the magnesium sulphate such salts as KNO_3, $NaNO_3$, KBr, NaCl, NH_4Cl, KI, NaI, $NaNO_2$, in varying percentages (from .02 to .1 per cent). In every case I found that the variety of B. pyocyaneus producing pyocyanin only was able to produce its pigment. Thus, we see, as Jordan ('99) has already noted, that the production of pyocyanin is independent of both the magnesium and acid group "SO_4."

Taking next a medium containing $MgSO_4$, but no K_2HPO_4, as

(L.)	Asparagin	1.00 per cent.
	$MgSO_4$	0.02 " "
	Distilled water.	

I found that this same variety of B. pyocyaneus gave a slight tinge of blue. The varieties of pyocyaneus which, on bouillon and on the asparagin, magnesium sulphate, and phosphate, gave both pyocyanin and the fluorescent pigments were, on the other hand, absolutely colorless. By adding other salts (as KNO_3, KCL, $NaNO_3$, NaCl, NH_4Cl, NaBr, NaI, KBr, $NaNO_2$), to replace the potassium phosphate, I found that the nitrates and chlorides, though giving a fair growth, did not favor the production of pyocyanin; that the bromides, iodides, and nitrates gave even less pyocyanin — oftentimes, indeed, being colorless. When the potassium phosphate, however, was replaced by phosphates of sodium and ammonia there was an abundant formation of pyocyanin, though by no means as great as on the medium found more favorable for pigment production (namely, asparagin 1 per cent, $MgSO_4$, .02 per cent, K_2HPO_4, .1 per cent).

(2.) Conclusions regarding Pyocyanin.

It would seem from these experiments that the production of pyocyanin is independent not only of sulphates and phosphates but also of magnesium and potassium. We must admit, however, that the presence of $MgSO_4$ and some phosphates greatly increases the production of pyocyanin; and that, of the phosphates, potassium is the most favorable. Indeed, those varieties of B. pyocyaneus which produce pyocyanin only in small amounts, when either the phosphates or sulphates are left out, give a good production upon such a medium as M.

(M.)	Asparagin	1.0 per cent.
	Na_2SO_4	0.1 " "
	K_2HPO_4	0.1 " "

On this solution the varieties which produce both pyocyanin and the fluorescent pigments quickly form both of them. This brings us to an investigation of the fluorescent pigments which were not produced on media which did not contain both sulphates and phosphates.

(3.) Fluorescent Pigments.

When the sodium of the medium M was replaced by other bases, as ammonium or calcium, the potassium by sodium and ammonium, it was found that the fluorescent pigment could be formed whenever, in addition to asparagin, there was present in the solution both phosphates and sulphates, irrespective of the base. From asparagin one per cent, $(NH_4)_3 PO_4$, and $(NH_4)_2SO_4$, most of the fluorescent bacteria produced their pigment, though not as well as when $MgSO_4$ and K_2HPO_4 were present. The results of the experiments on the production of pyocyanin and fluorescent pigments thus far agree in the main with the conclusions of Jordan, that for the production of blue-green fluorescent pigments both sulphates and phosphates must be present, while pyocyanin, on the contrary, can be formed on other combinations and still simpler solutions. Upon the various media used thus far, the action of other chromogenic bacteria than those producing pyocyanin and fluorescent pigments has been somewhat uncertain. Upon most of the media which proved favorable to the production of the two kinds of pigment just mentioned, the red, violet, black, and yellow pigments either appear only as a faintly perceivable line at the top or not at all.

(4.) The Pyocyanin and the Fluorescent Functions.

Before turning to the other pigments, however, let us examine more closely the pyocyanin and the fluorescent functions. Since Gessard's ('82) discovery of a bacillus which produced a blue or blue-green pigment, soluble in chloroform, many experiments have been carried on, not only as regards the morphological character of the bacillus to which Gessard gave the name of B. pyocyaneus, but also to the nature of its pigments. In the study of B. pyocyaneus many varieties have been isolated, so that at present we have kinds which produce pyocyanin alone, others which produce both pyocyanin and a yellowish-green fluorescent pigment, insoluble in chloroform but soluble in alcohol and ether, and further, some perhaps degenerate types, which produce a fluorescent pigment only. As Jordan ('99) says, attempts to determine the nature of the pigments and the conditions under which the chromogenic property is manifested have not led to concordant results. At one extreme we find an investigator, Thumm ('95), who maintains that only one pigment is formed by the different races or varieties, and that this pigment is identical with the ordinary blue-green fluorescent substance produced by the growth of common water bacteria, and who is also of the opinion that the several "races" differ chiefly in their power to produce ammonia, which, like other alkalis, modifies the color of the fluorescent pigment. On the other hand, other observers claim that one and the same variety of B. pyocyaneus is able to produce as many as four distinct pigments, black, blue, green, and yellow, at the same time and in the same culture media. The first of these theories is now held to be incorrect, since at least two pigments can easily be distinguished in a normal variety of B. pyocyaneus. From these two pigments, a blue, soluble in chloroform, and a bluish-green fluorescent pigment insoluble in chloroform but soluble in alcohol and ether, we can derive by oxidation the black from the blue, the yellow from the blue-green fluorescent pigment. Considering that B. pyocyaneus in the majority of cases does produce two pigments, the question is what is the relation between these pigments? Are the varieties characterized by the ability to produce different pigments, or can the same bacteria be compelled to form different colored products according to the medium on which they are grown? That the latter view is the correct one would seem to be the conclusion from the following experiments:

(*a.*) Gessard's Theory.

One and the same variety gives different colored pigments on different media, according as the media are alkaline or acid, or contain much or little of the phosphates. Gessard ('92) had noticed that the pigments were somewhat variable and he performed some experiments which led him to believe that the blue pyocyanin was produced whenever there was an excess of nitrogen over phosphates, and that the fluorescent pigment was produced whenever the phosphate content was high compared to the nitrogen.

(*b.*) Experiments on the Pyocyanin and the Fluorescent Functions.

Amplifying Gessard's experiments, I came to the following results, somewhat different from his. A variety which produced pyocyanin only on a medium (consisting of asparagin 1 per cent, $MgSO_4$ .02 per cent and K_2HPO_4 .1 per cent) was transferred to media in which the phosphate content varied from .001 per cent to .5 per cent. In the media containing low percentage of K_2HPO_4, pyocyanin alone was produced, while on the medium containing .5 per cent K_2HPO_4 both pyocyanin and the fluorescent pigment were produced. When the latter medium was rendered slightly acid, however, pyocyanin only was produced. When it was rendered slightly alkaline, on the other hand, little pyocyanin and much of the fluorescent pigment were produced. Another variety which was producing both pyocyanin and the fluorescent pigment (on asparagin 1 per cent, $MgSO_4$ .02 per cent, K_2HPO_4 .1 per cent) was made to produce the fluorescent pigment alone (on asparagin .2 per cent, $MgSO_4$ .2 per cent, K_2HPO_4 .5 per cent). This same variety (upon asparagin 1 per cent, $MgSO_4$ .05 per cent, K_2HPO_4 .2 per cent, strongly acid) produced pyocyanin alone. „Taking next a variety of B. pyocyaneus which had lost the power to produce pyocyanin but was, however, producing the fluorescent pigment, I placed it upon media in which the phosphates varied from .5 per cent to .001 per cent. In no case, no matter what the reaction, did pyocyanin appear. Bacteria producing pyocyanin cannot be made to produce fluorescent pigment.

Solutions were now made up in which the phosphates content was gradually lessened, and another in which the sulphates were decreased in percentages to determine whether or not the fluorescent bacilli, including that variety of B. pyocyaneus which was producing the fluorescent pigment only, could be induced to take up the pyocyanin function. The fluorescent pigment disappeared as the salt was lessened, but no pyocyanin was produced. In all of these experiments, I distinguished pyocyanin from the fluorescent pigment by the blue-green solution pyocyanin gives with chloroform. In chloroform the blue-green fluorescent pigment is insoluble. It was rather peculiar that after the extraction of a culture of B. pyocyaneus with chloroform the blue-green pigment could be seen forming later at the top of the layer, insoluble in chloroform, showing that the bacteria were growing in the stratum above the chloroform.

(*c*) Conclusions regarding the Pyocyanin and the Fluorescent Function.

The conclusions to be drawn are as follows:

A variety of B. pyocyaneus producing both pyocyanin and the fluorescent pigment can be made to produce pyocyanin alone, pyocyanin and a fluorescent pigment, or the fluorescent pigment alone, according to the medium upon which it is grown; the purely fluorescent bacteria, including the fluorescent variety of B. pyocyaneus, cannot be made to take up the pyocyanin function; a variety of B. pyocyaneus producing pyocyanin only cannot be made to take up the fluorescent function.

An alkaline reaction and a high phosphate content favor the production of the fluorescent pigment, while a low phosphate content and acid reaction favor the production of pyocyanin.

(*d.*) Oxidation Products from these Pigments.

Both of these pigments give oxidation products. The pyocyanin I have separated and kept for several months, at the end of which time it appeared black, but upon dilution showed itself brown-red. This brown-red pigment I consider to be identical with the brown-red pyoxanthose which Gessard ('90) secured from the blue pyocyanin by oxidation. The fluorescent pigment, on the other hand, originally blue-green, changes to a yellow on standing or in an acid solution. In these oxidation products we have, as Jordan has noted, the reason for the fact that so many have asserted — that B. pyocyaneus produces four pigments, namely, black, blue-green, and yellow.

(5.) Red and Violet Pigments.

The red and violet pigments treated here include the pigments produced by B. prodigiosus, B. rosaceus metalloides, B. ruber balticus, B. violaceus, and B. janthinus. M. roseus and M. mycoides roseus developed pigment only on media containing lactic acid. The latter pigment-producing bacteria I shall therefore consider when treating of the influence of the ammonium salts of organic acids. B. havaniensis and M. agilis, red pigment producers, either did not grow at all on non-albuminous media or produced pigment so slowly on such media that for the purpose of biochemical study none but albuminous material are of any use.

Taking then the red and violet pigment producers, which thrive on simple media, we inoculated them on the following medium, N:

(N.)	Asparagin	1.00	per cent.
	K_2HPO_4	0.1	" "
	$MgSO_4$	0.02	" "

All of these bacteria produced their characteristic pigments upon this solution, though in some cases very slightly.

If two per cent glycerin be added, however, there is abundant development of pigment in every case. Leaving the glycerin out of the solution, however, the $MgSO_4$ of medium N was replaced by other salts (as KNO_3, $NaNO_3$, KCL, NaCl, NH_4Cl, KBr, KI, NaI, $NaNO_2$, Na_2SO_4, and K_2SO_4, in percentages ranging from .02 per cent to .5 per cent). The iodides, bromides, and nitrates of all the metals, though allowing growth, did not give a pigment production as judged by the results of from three to five repetitions. The chloride of sodium gave better growth than the other chlorides. With the exception of a very faint line near the top of the Na_2SO_4 solution, B. prodigiosus, B. ruber balticus, B. ruber metalloides, B. violaceus, and B. janthinus did not produce any pigment when the $MgSO_4$ of the asparagin — K_2HPO_4 $MgSO_4$ media was replaced by other salts. Even upon asparagin one per cent, K_2HPO_4 .1 per cent, $Mg(NO_3)_2$ .02 per cent, no pigment appeared. Replacing the K_2HPO_4 of the medium containing asparagin one per cent, $MgSO_4$ .02 per cent, and K_2HPO_4 .1 per cent, by various salts other than phosphates gave very little growth and no pigment. On the other hand, replacing the potassium phosphate by other phosphates gave good growth and a slight formation of pigment. The phosphates of other metals than potassium did not prove as favorable for pigment production as the potassium salt. Indeed, the frequent transfer on phosphates of sodium and ammonium decreased the pigment to a hardly perceivable line at the top. Since upon this medium (consisting of asparagin one per cent, K_2HPO_4 .1 per cent, and $MgSO_4$ .02 per cent) the production of pigment was never very abundant, it seemed best to enrich the medium by a small amount of glycerin.

When to medium N one per cent of glycerin was added the reds and violets quickly formed their pigments. When the $MgSO_4$ was left out, however, there was no formation of pigment, though the growth was good. When the $MgSO_4$ was replaced by other sulphates, as of sodium and potassium, a very faint ring appeared at the top of the solutions, but this ring I attribute to the presence of a trace of $MgSO_4$ in the glycerin. To a solution (consisting of asparagin one per cent, glycerin one per cent, K_2HPO_4 .1 per cent), there was added from .02 to .1 per cent of each of the following salts: NaBr, K_2S, Na_2S, NaCl, KCl, KNO_3, $NaNO_3$. On the bromides, sulphides, and iodides no pigment appeared. In the chloride and nitrate a very faint ring of pigment appeared, but as this was not constantly produced I attribute the pigmentation to the presence of traces of $MgSO_4$ as impurities in the salts or taken from the glass tubes. With one per cent of glycerin in the solution Na_2HPO_4 in place of K_2HPO_4 gave a good production of pigment. When agar or gelatin was used as a base, the results were by no means in agreement with those found in liquid media At first I thought the better production on simple media with the colloids was due to a greater aeration produced by the long slant. On replacing the agar and gelatin by colloidal silicic acid to get rid of the impurities of agar and gelatin, I found that the results on the slant culture of silicic acid were in exact agreement with those based on liquid culture. From these experiments it is seen that a phosphate and $MgSO_4$ must be present for the production of the pigments of B. prodigiosus, B. ruber balticus, B. rosaceus metalloides, B. violaceus, B. janthinus. The very slight shade of pigment produced by Na_2SO_4 I believe to be due to an impurity of $MgSO_4$, since, as will be later shown, a very small trace of the latter salt suffices for the production of pigment.

Concluding that $MgSO_4$ and K_2HPO_4 were necessary for the production of these pigments, I made a series as follows, to see how widely the percentages may vary and still give a good production of pigment.

(O.)	Asparagin	1.00	per cent.
	$MgSO_4$	0.001–0.1	" "
	K_2HPO_4	0.1	" "
(P.)	Peptone	1.00	" "
	$MgSO_4$	0.1–16	" "

The pigments were still slightly produced in the lower percentages. Above two per cent no pigment was produced.

On medium

(Q.)	Asparagin	1.00	per cent.
	$MgSO_4$	0.02	" "
	K_2HPO_4	0.001–0.5	" "

in every case pigment was produced, but better in the medium percentages.

From the preceding experiments it can be seen that exceedingly small amounts of $MgSO_4$ and K_2HPO_4, especially of $MgSO_4$, will suffice for the production of pigment. With the omission of either of these salts, especially of the $MgSO_4$, the pigment production immediately falls to a slight, hardly perceptible ring at the top of the solution. Though I formerly thought that these pigments could be produced whenever there was present — in addition to suitable compounds of carbon, hydrogen, oxygen, and nitrogen — phosphates, together with sulphates, sulphites, chlorides, or nitrates (Sullivan, '03), I now believe that for the formation of the reddish pigments of B. prodigiosus, B. ruber balticus, B. rosaceus metalloides for the violet pigments of B. janthinus and B. violaceus, there must be present $MgSO_4$ and a phosphate, preferably K_2HPO_4, in addition to suitable compounds of carbon, hydrogen, oxygen, and nitrogen. Küntze ('00) in his work on the pigment of B. prodigiosus declared that $MgSO_4$ alone was absolutely necessary. Since he concludes, however, that K_2HPO_4 is necessary for the life of every living cell, his conclusions and mine as regards the chemical requirements of B. prodigiosus are practically the same.

(6.) Yellow Pigments.

The yellow pigments produced by S. lutea, M. citreus, S. pyogenes citreus, M. tetragenus versatilis, S. aurantiaca, B. fuscus, and M. cereus flavus cannot be formed to any extent on non-albuminous material. Accordingly, to find the conditions under which these yellow pigment-producing bacteria formed their pigment, we had recourse to a one per cent peptone solution. Upon a solution containing nothing but peptone, S. lutea, S. aurantiaca, M. citreus, M. tetragenous versatilis, S. pyogenes citreus, M. cereus flavus, and M.

agilis grew and produced a slight trace of pigment. Commercial peptone has been found, by a recent investigation, to contain phosphates and sulphates, and other salts in slight traces. These traces I tried to remove, first by dialyzing peptone in a parchment tube for from ten to sixty hours. In a one per cent solution of dialyzed peptone, M. citreus, S. pyogenes citreus, A. aurantiaca, quickly developed their color. M. cereus flavus, M. tetragenous versatilis, S. lutea and M. agilis did not grow at all. On adding one per cent Na_2SO_4 to the solution, S. lutea gave a good yellow sediment, while M. cereus flavus, M. tetragenous versatilis were colorless and M. agilis did not develop at all. On adding one-tenth of one per cent K_2HPO_4 to the peptone — sodium sulphate solution — M. cereus flavus also developed its characteristic pigment; $MgSO$ acted like the Na_2SO_4.

Little can be said regarding the salts that are necessary for the formation of the yellow pigments mentioned above, since it was found impossible to free the peptone from impurities either by precipitation or by dialysis. It appears, however, that the presence of $MgSO_4$ and K_2HPO_4 in the peptone solution facilitates the production of the pigment and gives a more vivid color.

An attempt was made to adapt the yellow organisms to a nonalbuminous medium. Starting with a medium consisting of

Asparagin	0.5	per	cent.
Peptone	0.5	"	"
$MgSO_4$	0.02	"	"
K_2HPO_4	0.1	"	"

upon which all the yellow pigment producers formed their pigment, the peptone content was gradually reduced to ·0005 per cent. As the peptone diminished in amount, the pigment was progressively slower in appearing, until on a practically nonproteid medium it was many weeks in forming. I have no doubt, however, that most of the yellow pigment producers can be adapted to nonproteid media. Concerning this subject, however, I shall have more to say in a later paper.

(C.) The Ammonium Salts of Organic Acids.

As regards the value of the organic acids for pigment production, Lepierre ('95), who studied the behavior of a species closely related to B. fl. putridus in solutions of various ammonium salts, drew from his work the conclusions that the fluorescence is intimately bound up with: first, the bibasic character of the acid; second, the existence in the molecule of at least two groups of CH_2; Jordan ('99), on the other hand, in his work on fluorescent bacteria, was able to utilize the urate of ammonium, which possesses neither carboxyl (COOH) nor the methylene (CH_2) grouping.

In order to test the availability of the organic acids for pigment production, I employed the ammonium salts of succinic, lactic, citric, malic, tartaric, oxalic, uric, acetic, and formic acids, in amounts varying from .05 per cent to .5 per cent. To solutions containing some one of these salts, $MgSO_4$ (.02 per cent) and K_2HPO_4 (.1 per cent) were added. The succinate gave the best production of pigment, the lactate and the citrate solutions next. In the case of the malate, tartrate, oxalate, however, no pigment appeared though there was more or less growth. Contrary to the observation of Jordan ('99) as regards the production of fluorescent pigments, I perceived no pigment formation on the urate, acetate, or formate, no matter whether the concentration of salts was .05 per cent or .5 per cent. Contrary to Lepierre, I found that citric acid was especially favorable for pigment production, especially for such varieties as B. roseus, M. mycoides roseus, and the blue-black pigments of cyaneo fluorescens, which hitherto had shown very little pigment on the nonalbuminous media — as in the case of B. roseus and M. mycoides roseus, or not at all — as in the case of the blue-black pigment produced by B. cyaneo fluorescens. Accordingly, making a solution, R,

(R).	Ammonium lactate	0.4	per cent.
	$MgSO_4$	0.02	" "
	K_2HPO_4	0.1	" "

I replaced the $MgSO_4$ by other salts, as $NaCl$, Na_2SO_4, with the result that M. roseus and M. mycoides roseus still produced pigment slightly. The black ring given by cyaneofluorescens, on the other hand, appears only when magnesium sulphate is present in addition to lactic acid.

As regards the availability of the organic acids, I should agree in the main with Lepierre that the presence of carboxyl ($COOH$) and the methylene group (CH_2) is coincident with superior nutritive value and chromogenic power.

(D.) Dubois' Theory of Isotonic Solutions.

Dubois ('98) claims that the various salts which bacteria require are not nutriment, but are necessary to render the solutions isotonic, that is, to keep the surrounding liquids in a proper state to preserve the bioprotein of the bacteria in a certain state of hydration. Sugar in sufficient quantities, he says, will give the same results. Work on pigment production, however, does not corroborate Dubois' assertion, for though sugar solutions from one one-hundredth to one per cent were employed in place of $MgSO_4$ and K_2HPO_4, no pigment was found. The addition of $MgSO_4$ and K_2HPO_4 to the sugar-asparagin solution, however, speedily give a good formation of pigment in the case of the violets and reds. These salts are undoubtedly used as foods directly, or to offset the poisonous effects of the acids produced by the bacteria. The function of these salts, however, we shall discuss in greater detail in the following section.

(E.) The Physiological Role of Mineral Nutrients.

The functions of the mineral nutrients in plants and animals constitute a highly important problem. As Loew ('99) points out, the mineral salts have not only to perform specific chemical functions, but also seem to contribute directly to the maintenance of the living condition of the protoplasm. A most striking instance of this is the rapid dying of infusoria in distilled water. Years ago M. Nencke (Loew, '99) recognized the importance of the mineral matter combined with the proteids. "All proteids," he says, "occurring in the living protoplasm are combined with mineral substances, whereby the proteins concerned acquire specific properties and functional significations in the organisms." As to the physiological value of potassium Loew ('99) shows that it has a greater condensation power than other salts and consequently is of greater use to plants in synthetizing their protein molecules.

In studying the questions as to what the nourishing function of magnesium salts is and why they cannot be replaced physiologically by calcium salts, the probable answer is found, according to Loew ('99), in the well-known property of magnesium salts to easily undergo dissociations. In the assimilation of nitrogen from nitrates or amines, sulphur from sulphates, and phosphoric acids from phosphates, the dissociation of these salts would immediately precede assimilation; hence the easier these acids are separated from the base the easier their assimilation will be accomplished. The fact that magnesic sulphate and potassium hypophosphate are of so much use to bacteria in their growth and pigment formation is of great importance as regards their relation to higher plants. As Strasburger ('98) says, "sulphur and phosphorus form, like nitrogen, important constituents of protoplasm. The sulphur is taken into the plants in the form of phosphates. Magnesium, like potassium, participating in the most important synthetic processes of plants, is found in combination with various acids." The addition of other salts (such as Na_2SO_4, NaCl, KNO_3, $CaSO_4$) to a nutrient medium for chromogenic bacteria exerts an active and generally beneficial influence. All these salts are undoubtedly of use in aiding osmosis, in neutralizing hurtful by-products, and in affording a better means of chemical synthesis through their SO_2, NO_3, Cl, PO_4 groups. Of the bases, magnesium and potassium are of greater value for chemical synthesis, especially in the case of chromogenic bacteria — the former because of its greater plasticity and the readiness with which it can be dissociated; the latter because of its greater condensing power.

The organic salts, such as the lactates, citrates, etc., on the other hand, aid in chemical synthesis by affording organic groups on which the synthesis may be made.

(F.) Influence of Light and Heat on Pigment Production.

Laurent ('90), Dieudonné ('94), Beck and Schultz ('96), and Mary Hefferan ('04) all found that direct sunlight not only hindered pigment production but retarded growth as well. If the action of the direct sunlight is prolonged, there results a generation of weaker organisms — shown, for example, by incomplete liquefaction, slight production of pigment, lessened virulence — which only regain their original properties by repeated transplantation or fresh media. With still longer action of the direct sunlight, Laurent ('90) made a series of experiments and found that five hours of sunlight not only destroyed the color but also sterilized the media; three hours gave colored and colorless growths, and the colored gradually became colorless; one hour gave colored and colorless, but the colorless colonies became colored in diffused light. Others he found never regained the power of pigmentation, even upon the most favorable media. I have made no experiments with regard to the action of direct sunlight, but have made a comparison of cultures kept in the dark with others kept in diffused daylight. Both cultures were kept at the same temperature with slight variations, and in the main, those cultures that were kept in the dark showed a slightly more vivid color. Pigments grown in the dark gradually fade in the light — due, probably, to the oxidizing effect of the light, since the same effect is noticed when dilute potassium permanganate is added to the solution containing the pigment. According to Migula ('97) light is unfavorable to all bacteria in varying degrees, and all bacteria thrive better in the dark. Certain chromogenic bacteria, however, as M. ochroleucus (von Prove) (Flügge, '96), forms its pigment only in the light. Though light has some slight effect on the production of pigment, heat, on the other hand, has a great influence on the formation of bacterial colors. Thus B. prodigiosus, B. rosaceus metalloides, B. ruber balticus, B. violaceus, B. janthinus, which on certain media produce an abundance of pigment, grew on the same media at a temperature of 40° C. without the slightest trace of color. By transplanting from these colorless varieties to the media at room temperature the bacteria regained their power of forming pigment almost immediately. If the inoculations are carried on at 40° C., for several weeks, the power to produce pigment at the room temperature is gradually diminished, and in some cases never returns. Indeed, Schotteleus ('87) found that by long-continued development at 37° C., B. prodigiosus could be converted to a non-chromogenic variety which never regained its power of forming its characteristic carmine color. Others have found that B. cyanogenes and B. pyocyaneus behave the same way. The loss of color at high temperatures is due, most probably, to a lessening of the vital activities of the organism, since antiseptics, as carbolic acid, hydrochloric acid, tartaric acid and potassium permanganate also cause the bacteria to grow colorless. Infrequent transfers from cultures deficient in water also lead to a diminishing of the pigment production, and sometimes the bacteria lose their chromogenic power altogether. Occasionally, however, we find, even upon a newly made agar streak, some colonies producing color, others absolutely colorless. These colorless varieties are undoubtedly "sports" — examples of miscontinuous variation, arising from some internal condition which makes a change in the sum total of the environment. These "sports" indeed may be perpetuated. R. O. Neumann ('97) has by selection grown from M. pyogenes aureus, white, yellow, and red varieties. H. W. Conn ('00) more recently has described natural varieties of a certain chromogenic bacterium, varieties which he was able to keep distinct one from another by selection.

(G.) The Influence of the Reaction of the Medium.

Slight changes in the reaction of the media either alkaline or acid have little effect upon the production of pigment. Greater variation one way or another, as Wasserzug discovered, leads to a colorless growth which at times is extremely abundant. The interference of the acid, at least, seems to be rather with the metabolic activities of the bacteria than with the pigment, as the following facts indicate: The pigment when once formed is either rendered colorless or changed in tint by acid, but the addition of alkali brings it back as vividly as ever. The process of acidification and neutralization can be repeated a number of times without weakening the intensity of the pigment.

The influence of acid is well shown by a medium containing one per cent dextrose. B. pyocyaneus, B. fluorescens liquifaciens, B. prodigiosus, B. janthinus, and B. violaceus soon formed a slight pigment which, however, in a few days is not perceivable in the mass of colorless growth. No color appears even when alkali is added at this stage, conclusively showing that the presence of acid interferes with the pigment production and does not simply mask its presence. After a growth of five to fifteen days all of the bacteria mentioned above excepting the B. fluorescens liquefaciens, which does not form its pigment in acid solutions, showed pigment. On this medium then there is a struggle between two of the vital activities of organisms, namely, the ability to produce acid and the power of forming alkali. The latter function ultimately gains the upper hand. B. pyocyaneus on this medium was yellowish at first even after shaking, but later, when the growth was very heavy the colorless solution on shaking gave a beautiful robin's egg blue, showing that there was a presence of leuco-body which, with the oxygen of the air, formed the typical blue pyocyanin. The effect of the production of alkali is shown in the ordinary growth of the bacteria. At first the red pigments are somewhat pink, but later under the influence of the alkali formed they deepen and become more vivid. The violet pigments likewise change to a deep black purple.

(H.) The Influence of Oxygen.

As shown in the preceding section B. pyocyaneus produces a colorless substance which on shaking becomes a robin's egg blue. All the chromogenic bacteria excepting anaërobic S. rubrum develop their pigment only when there is free access of air. When the air is kept out by a film of oil the color does not appear, although there is abundant growth. Ledderhose ('88) supposed that the bacteria formed leuco-bodies and that with the free access of oxygen the leuco-body gave colored products. It would seem from the preceding experiment performed first by Liborius ('86) that Ledderhose's supposition is true.

4. *Other Metabolic Products of Bacterial Growth.*

A. Pigments and Albuminous Bodies.

With the free access of oxygen, the chromogenic bacteria thrive well, and on nonalbuminous media give rise to various metabolic projects. Of these, the most conspicuous is the pigment. These pigments are precipitated by the heavy metals as mercury, lead, and by $(NH_4)_2SO_4$. Thus they act somewhat like albuminous bodies. A fluid culture of B. pyocyaneus, B. prodigiosus, B. ruber balticus, B. violaceus, and B. janthinus, on proteid free media, gives a strong reaction with Millon's reagent, thus showing the presence either of tyrosine or an (OH) derivative of benzol. Further, all these cultures give the xanthoproteic and Adamkiewicz reaction for albumens. A culture filtered through porcelain still gives the test for albumens, showing that the bacteria can synthesize albumen from very simple solutions. The result is the same as regards the test for albumens, whether the chromogenic variety are grown colorless or colored. Control solutions did not give the test for albumens.

Since it is practically impossible to secure the pigments in a pure state, no attempts were made to learn their spectroscopic appearance or their empirical formulæ. Ledderhose ('88), however, isolated the blue-green pyocyanin and determined its formula to be $C_{14}H_{14}N_2O_4$, Fordos ('60), on the other hand, asserts that pyocyanin also contains sulphur. Griffiths ('92) has determined the formula of the pigment produced by B. prodigiosus to be $C_{38}H_{56}NO_5$.

B. Acid Production.

Aside from the production of bodies which react to the standard tests for albumen, there is produced by B. prodigiosus, B. ruber balticus, B. rosaceus metalloides, B. violaceus, B. janthinus, and B. pyocyaneus first acids and then ammonia. The acids produced by these bacteria I have analyzed by the method given by Chester ('01) in his "Manual of Determinative Bacteriology," page 39. By this method I tested for formic, acetic, propionic, butyric, and lactic acid. To determine the citric, tartaric, and oxalic acids, and to verify the results by Chester's method, I employed Prescott and Johnson's "Qualitative Chemical Analysis," 5th edition, and Fresenius' "Manual of Qualitative Analysis," 1902. Since the tests for the organic acids given in the text-books seem to be of questionable value when applied to mixtures, I am reporting here only those acids which I am sure are present. B. prodigiosus, in addition to a volatile aromatic body resembling methyl amine in odor, was found to have in the medium a slight amount of citric and acetic acids and much formic acid; B. rosaceus metalloides, acetic acid; B. ruber balticus, formic; B. violaceus and B. janthinus, formic; B. pyocyaneus, sulphuretted hydrogen, mercaptan, and formic acid.

Making now a comparison of a colorless and a colored growth of B. prodigiosus on a simple solution of asparagin and other salts, I found that the production of acids was the same in both cases as far as analyzed, thus showing that the acid production is independent of the formation of pigment, but is rather a result of the life activity of the bacteria, which by acting on the asparagin and sugar, and breaking them down to lower compounds, secure the necessary energy to build up the protoplasm of their bodies, and, when the proper salts are present, to aid in the synthesis of pigments.

C. The Synthesis of Pigment and Albuminous Bodies by Bacteria.

How the bacteria build up their protoplasm it is hard to say. Loew ('91) declares that in bacterial synthesis formaldehyde is the primary assimilation product. From formaldehyde, sugar is formed by polymerization. This sugar, with the aid of combinations containing sulphur or nitrogen, is synthesized to albuminoids. In the same way, according to Loew, we may expect that all chemical bodies which contain ready-made formaldehyde, or are able to form it by simple rearrangement of the atoms, can serve also as material for the synthesis of carbohydrates and proteids. Thus asparagin ($COOH, CHNH_2, CH_2, CONH_2$), which is the amide of amido-succinic acid, contains nitrogen, which can be utilized in the same way as ammonia. It also contains the raw material of formaldehyde (CH_2O), and can be built up into a proteid with the aid of mineral salts. Thus Loew has shown that the amido-succinic acid, from which asparagin is derived, can be synthetized to a high complex molecule:

$$3C_4H_7NO_2 + \text{protoplasm} = C_{12}H_{17}N_3O_4\,{*} + 2H_2O$$

(amido-succinic acid).

$$6C_{12}H_{17}N_3O_4 + 6H_2 + H_2S = C_{72}H_{112}N_{18}SO_{22} + 2H^2O.$$

$C_{72}H_{112}N_{18}SO_{22}$, according to Loew, is the simplest expression for proteid. Whether or not the albuminous material is built up from formaldehyde by polymerization and synthesis or from asparagin, it is somewhat significant that formic acid was found in the products of the chromogenic bacteria which I tested. We might note further that Hueppe ('87) found that the organisms of nitrification developed their organic substance out of ammonium carbonate, and that in the synthesis of the body protoplasm from the carbonate, formaldehyde was formed.

In considering the synthesis of proteid matter, Schutzerberger ('88) believes that in order to effect such a synthesis, it is necessary to combine a molecule of leucine (an amido fatty acid) with a molecule leuceine (an amido acid of the acrylic series) with the elimination of water and then to combine this complex group with one or more molecules of urea and oxamid. His experiment was as follows: leucine and leuceine were mixed with about ten per cent by weight of urea and fully pulverized. The mixture was dried at 110° C., and intimately mixed with 1.5 times its weight of phosphoric anhydride and heated in an oil bath. At 120° C., there was no change, but at 125° C., dehydration took place very rapidly and the mixture became pasty. Soon it solidified into a compact product without darkening. This product was purified and behaved in many ways like an albuminous body.

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* I hexylamido-benzol would have this formula.

If Schutzerberger could approximate a proteid body by artificial means, how much more easily should living protoplasm bring about the same results?

Schulze (Escombe, '98), on the other hand, holds that the amido acids and bases are decomposed with the formation of nitrogenous residues which unite with non-nitrogenous compounds to form the amides synthetically. The latter are to be regarded as the material from which the proteid of growing plants are produced. In connection with Schulze's theory of the combination of nitrogenous material with non-nitrogenous, it is interesting to notice that the addition of sugar or glycerin to an asparagin salt solution gives a quicker growth and a better development of pigment. In the case of bacteria we find they are able to synthesize from simple to complex, and that having once started a certain line of synthesis, with the same conditions, the same line persists, with different conditions another pathway of anabolism is formed. Thus bacteria producing pigment at 20° C. no longer develop pigment at 37° C. My view of this synthesis of albuminoid matter by pigment-producing bacteria is that the living protoplasm acting as an enzyme and aided by groups, such as SO_4 and PO_4 and by plastic bases, as magnesium, effects a synthesis of the albuminoid matter with the formation of various by-products, as ammonia, acids, colored and toxic bodies.

As regards the foundation of pigment by bacteria, we may safely conclude that it is merely an incidental product of the metabolic activities. Thus bacteria ordinarily non-chromogenic may at times display vivid colors. B. pneumoniæ and B. diphtheriæ at present in our stock cultures have produced pigment, — the one a pink, the other a yellow. It is not at all strange, indeed, that colored bodies should arise in the vast complex of the metabolic products of bacteria. A pathway of synthesis which gives rise to pigment being once firmly established, this pathway persists under ordinary conditions. The pigment thus formed may or may not be of use to the organism. In higher organisms it is undoubtedly true that coloration in many cases is of great value to the organism possessing it from a biological standpoint. Taken all in all, however, the formation of pigment is incidental. The fundamental question is — Why do certain arrangements of atoms produce molecules which are colored; and not, Why does an organism under certain conditions produce pigment?

SUMMARY AND CONCLUSIONS.

The results of these experiments may be summarized under the following heads:

1. The view cannot longer be held that a sharp line of demarcation exists between chlorophyll-containing plants and those devoid of chlorophyll, since bacteria, like the higher plants, can build up their protoplasm and albuminous products, and their complex pigments, from very simple solutions.
2. The blue-green pyocyanin is formed whenever there is present a suitable combination of carbon, hydrogen, oxygen, and nitrogen, and some salt or salts to aid the synthesis or polymerization.
3. The production of pyocyanin is independent of the presence of either a phosphate or a sulphate.
4. For the formation of the fluorescent pigment, the presence of both sulphur and phosphorus is essential.
5. The nature of the base associated with the sulphur and phosphorus is not important.
6. The pyocyanin and the fluorescent functions are closely related, since, according to the medium in which it is grown,

one and the same variety of B. pyocyaneus can be made to produce pyocyanin alone, pyocyanin and a fluorescent pigment, or a fluorescent pigment alone.

7. The purely fluorescent bacteria cannot be made to take up the pyocyanin function. Bacteria producing pyocyanin alone cannot be made to take up the fluorescent function.

8. The fluorescent pigment formed by some varieties of B. pyocyaneus is produced under the same conditions as the fluorescent pigments in general.

9. The production of the fluorescent pigment is favored by a high phosphate content and a slightly alkaline reaction.

10. The production of pyocyanin is favored by a low phosphate content and a slightly acid reaction.

11. The red pigments produced by B. prodigiosus, B. rosaceus metalloides, B. ruber balticus, and the violet pigments of B. violaceus and B. janthinus require the presence of magnesic sulphate and a phosphate, preferably potassium phosphate.

12. The rose-red pigment of M. roseus and M. mycoides roseus, and the black pigment of B. cyaneo-fluorescens are produced only in the presence of lactic acid.

13. The yellow pigments produced by B. fuscus, M aurantiaca, M. Citreus, M. tetragenus versatilis, S. lutea, and M. cereus flavus, the red pigments of B. havaniensis and M. agilis, are formed very slowly in non-albuminous media.

14. These pigments are quickly formed in a peptone solution plus salts. As the peptone cannot be purified little can be said of the physiological value of these salts. The presence of magnesium sulphate and dipotassium phosphate, however, favors the production of these pigments.

15. Of the organic salts of ammonium, the malate, tartrate, and oxalate, in percentages not greater than four-tenths of one per cent, allowed growth but produced no pigment; the formate and acetate gave no pigment in any percentages. The lactate, citrate, and succinate, on the other hand, favored a good production of pigment.

16. The conclusion that may be drawn regarding the dependence of chromogenesis upon the molecular constitutions

of the ammonium salts may best be appreciated through an examination of the constitutional formulæ of the organic acids whose salts were employed. The list is arranged, so far as possible, in order of their ability to form pigment productions.

Asparagin	$\mathrm{COOH, CHNH_2, CH_2, CONH_2.}$
Succinic acid	$\mathrm{COOH, CH_2, CH_2, COOH.}$
Lactic acid	$\mathrm{CH_3CHOH, COOH.}$
Citric acid	$\mathrm{COOH, C(OH), (CH_2COOH)_2.}$
Malic acid	$\mathrm{COOH, CH_2, CHOH, COOH.}$
Tartaric acid	$\mathrm{COOH, CHOH, CHOH, COOH.}$
Oxalic acid	$\mathrm{COOH, COOH.}$
Acetic acid	$\mathrm{CH_3, COOH.}$
Uric acid	$\mathrm{NH, CO, NH, CO,\cdot C\text{-}CNH, CONH.}$
Formic acid	$\mathrm{H, COOH.}$

The first four allow the production of pigment when they are combined with a base such as ammonium. The next three allow growth but no pigment formation. The last three I found unfavorable both to growth and pigment production. Lepierre ('95), who studied the behavior of a species closely related to B. fl. putridus, draws from his work the conclusions that the fluorescence is intimately bound up with: first, the bibasicity of the acid; second, the existence in the molecule of at least two groups of CH_2. Jordan ('99), on the other hand, in his work on fluorescent bacteria was able to utilize the urate of ammonium, which possessed neither carboxyl ($COOH$) nor the methylene (CH_2) grouping. In my investigations, however, I have never observed pigment on the urate nor indeed on the acetate. Accordingly, I should agree with Lepierre that the presence of the carboxyl ($COOH$) and the methylene (CH_2) group is coincident with superior nutritive value and chromogenic power.

17. The salts which favor the production of pigment are necessary, not to render the solution isosmotic but either as direct nutriment for the bacteria or to fix the acids produced, which otherwise would have a toxic effect upon the bacteria, or to afford material for chemical synthesis.

18. The formation of pigment is dependent also upon the reaction of the media, suitable ranges of temperature, and

the free access of oxygen. Abnormal temperature, acid or alkaline media, and lack of oxygen lead to a colorless growth.

19. Beside the pigments and albumen-like bodies the chromogenic bacteria form acids and ammonia. These products apparently arise from the breaking up of asparagin or sugar and are independent of pigment formation.

20. The production of pigment is not an essential, vital act. As it is of no discoverable advantage to the organism possessing the power of producing it, its production is purely incidental.

BIBLIOGRAPHY.

PART I.

Synthetic culture media.

1786. Müller, O. F. Animalcula infusoria, fluviatilia et terrestria.

1848. Nägeli, O. Gattungen einseliger Algen. Migula's System der bakterien, i, 9.

1878. Schulze, E. Ueber Zersetzung u. Neubildung v. Eiweisstoffen in Lupinenkeimlingen Landwirt. Jahrb., vii, 411.

1880. Schulze, E. Ueber d. Eiweissumsatz in Pflanzenorganismus. Landwirt. Jahrb., ix, 689.

1886. Bolton Meade. Ueber das Verhalten verschiedener Bacterienarten in Trinkwasser. Zeitschr. f. Hygiene, i, 76.

1887. Hueppe, F. Ueber Chlorophyllwirkung Chlorophyllfreier Pflanzen Biol. Centralbl., vii, 701.

1888. Schutzenberger, P. M. Recherches sur la synthèse des matières albuminoides et protéiques. C. R. de l'Acad. des Sci., cvi, 1407.

1890. Winogradsky, S. Recherches sur les organisms de la nitrification. Ann. de l'Inst. Pasteur, iv, 213, 257, 760.

1890. Jordan, E. O., and Richards, Ellen H. Investigations upon nitrification and the nitrifying organisms. Mass. State Board of Health. Report. Pin. S. and W., 863.

1890. Fermi, C. Die Leim u. fibrin-lösenden u. die diastatischen Fermente der Mikro-organismen. Centralbl. f. Bakt. u. Parasitenk., vii, 468.

1890. Kühne, W. Kieselsäure als Nährboden für Organismen. Zeitschr. f. Biologie, xxvii, N. F., ix, Heft., i, 171.

1890. Loew, O. Ueber das Verhalten niederer Pilze gegen verschiedene anorganische Stickstoffverbindungen. Biol. Cent., x, 577.

1891. Seleskin, P. Die Kieselsäuregallerte als Nährsubstrat. Centralbl. f. Bakt., x, 209.

1891. Schutzenberger, P. M. Essai sur la synthèse des matières proteiques. C. R. de l'Acad. du Sci., cxii, 198.

1891. Loew, O. Die Chemischen Verhältnisse des Bakterienlebens. C. f. B., ix, 690.

1891. Winogradsky, S. Recherches sur les organismes de la nitrification. Ann. de l'Inst. Pasteur, v., 93, 575

1892. Fermi, Cl. Beitrag zum Studium der von den Mikro-organismen abgesonderten diastatischen u. Inversionsfermente. Aus dem Hygienischen Institut der Kön Universität in Rom. C. f. Bakt. u. Parasitenkunde, xii, 713.

1892. Loew, O. Ein Beitrag zur Kenntniss der Chemischen Fähigkeiten. C. f. Bakt., xii, 361.

1892. Loew, O. Ueber einen Bacillus welcher Ameisensäure u. Formaldehyd assimilieren kann. C. f. Bakt., xii, 462

1893. De Schweinitz, E. A. Culture media for biochemic investigations. N.Y. Med. Journal, 1893.

1893. Uschinsky, N. Ueber eine eiweissfreie Nährsubstrat f. pathogene Bakterien nebst einigen Bemerkungen ueber Tetanusgift. Arch. de Med. experiment, 1893, No. 3.

1893. Winogradsky, S. Sur l'assimilation de l'azote gazeux de l'atmosphère par les microbes. Comptes Rendus de Paris.

1893. Mauthner, J. Ueber den Einfluss des Asparagin auf. den Umsatz des Eiweiss beim Fleischfresser. C. f. Physiol., 187.

1893. Politis, G. Ueber die Bedeutung des Asparagin als Nährungstoff. C. f. Physiol., 155.

1894. Fränkel, C. Beiträge zur Kenntnis des Bacterienwachsthums auf eiweissfreien Nährlösungen. Hyg. Rundschau, 769.

1895. Halliburton, W. H. The synthesis of proteids. Sci. Prog., iv, '95-'96, 149.

1897. Uschinsky, N. Ueber Diptheriekulturen auf eiweissfreier Nährlösung. C. f. Bakt. u. Parasitk, xxi, 146.

1897. Schulze, E. Ueber d. Umsatz d. Eiweisstoffe in d. lebenden Pflanze. B. f. Phy. Chem., xxiv, 18.

1897. Capaldi u. Proskauer. Beiträge z. Kenntnis der Säuerbildung bei Typhusbacillen u. B. coli. Zeitschr. f. Hygiene u. Inf., xxiii.

1898. Emmerich u. Loew. Die Ursache der künstlichen Immunität und die Heilung von Infektionskrankheiten. Münch. Med. Wochenschr., 1898, No. 40.

1898. Escombe, F. Germination of seeds. The hydrolysis and regeneration of proteins. Sci. Prog., vii, 219.

1899. Hueppe, F. The principles of bacteriology, p. 99. Translated by E. O. Jordan.

1901. Chester, F. D. A manual of determinative bacteriology, p. 44.

1902. Gran, H. H. Die Hydrolyse des Agars durch ein Enzym. Bergens Museums Aarbog., No. 2. C. f. B., ii, ix, 562.

1903. Beijerinck, M. W., u. Van Delden, A. Ueber eine farblose Bakterie, deren Kohlenstoffnahrung aus der atmosphärischen Luft herrührt. C. f B., ii, x, 33, 1903.

1903. Jacobitz, E. Beitrag zur Frage der Stickstoffassimilation durch den Bacillus ellenbochensis. Ztsch. f. Hyg., xlv, 96.

1903. Loew, O , u. Kozai, Y. Ueber Ernährungsverhältnisse beim B. prodigiosus (Enzymes). The Bul of the College of Agriculture, Tokio Imperial University, v, 137.

1904. Beijerinck, M. W. Ueber die Bakterien, welche sich im Dunklen mit Kohlensäure als Kohlenstoffquelle ernähren können. C. f. B., ii, xi, 593.

1904. Meldola, R. The chemical synthesis of vital products. London.

Part II.

Biochemistry of bacteria.

1860. Fordos, M. Recherches sur le native colorante des suppurations bleues; pyocyanin. C. R. de l'Acad. des Sci., lii, 215.

1866. Erdmann, O. Bildung von Anilinfarben aus Proteinkörpern. Jour. f. prakt. Chemie., iii, 385.

1869. Thudichum. Cent. f. d. Med. Wiss. Ref. M. Foster, Text-book of Physiology, Part V, appendix, 265.

1872. Schroeter, J. Ueber einige durch Bakterien gebildete Pigmente. Cohn's Beitr. z. Biol. der Pflanzen., i, Heft. 2, 109.

1875. Helm, O. Ueber Monas prodigiosa und den von ihr erzeugten Farbstoff. Archiv. f. Pharmacie, iii, 19.

1882. Gessard, C. De la pyocyanin et de son microbe. Thèse de Paris.

1882. Nägeli, C. Ref. De Bary Morphology and biology of fungi, mycetozoa and bacteria, 1884, 480.

1886. Liborius, P. Beiträge z. K. des Sauerstoffbedürfnisses der Bakterien. Zeit. f. Hyg., i, 115.

1887. Schotteleus, M. Biolog. Unters. über den Micrococcus prod. S. A. aus d. Festschrift für A. V. Kölliker, Leipzig.

1887. Wasserzug, E. Sur la formation de la matière colorante chez le Bacillus pyocyaneus. Annals de l'Inst. Pasteur, 1887.

1888. Ledderhose, G. Ueber den blauen Eiter. C. f. B., iii, 581.

1889. Frick, A. Green pigment bacilli. Virchow's Archiv., cxvi, Heft. 2, Baumgarten, 1889, 462.

1889. Hopkins, D. C. Pigment and yellow butterflies. Proc. Chem. Soc., v, 117.

1889. Kuebler, P. Ueber das Verhalten des M. prod. in sauer Fleischbrühe. C. f. Bakt., v, 333.

1889. Zopf, W. Ueber Pilzfarbstoffe. Bot. Zeit., xlvii, 85.

1889. Zopf, W. Vorkommen eines Lipochroms. bei Spaltpilzen. Bot. Zeit. ?

1889. Babes, A. Note sur quelques matières colorantes et aromatiques produites par le bacille pyocyanique. Comps. rendus Biol., 438. C. f. B., vi, 442, Baumgarten, 1889.

1890. Gessard, C. Sur les pigments divers produits par le microbe pyocyanique. La Semaine Med., 1890, No. 9, p. 67. Abst., C. f. B., vii, 508.

1890. Laurent, E. Etude sur la variabilité du bacille rouge de Kiel. Ann. de l'Inst. Pasteur, iv, 465.

1891. Beyerinck, M. W. Die Lebensgeschichte einer Pigment bacterie. Bot. Zeit., 1891.

1892. Gessard, C. Sur la fonction fluorescigène des Microbes. Ann. de l'Inst. Pasteur, vi, 801.

1893. Poulton, E. B. The experimental proof that the colors of certain lepidopterus larvæ are largely due to modified plant pigments derived from food. Proc. Royal Soc., London, liv, 417.

1893. Urech, F. Beiträge zur Kenntnis der Farbe von Insectengruppen. Zeitschr. wissenschaft. Zool., lvii, 306.

1893. Zopf, W. Zur Kenntnis der Farbungsursachen niederer Organismen. Zopf. Beiträge zur Physiol. u. Morphol. niederer Organismen, 1893, Heft. 3.

1894. Dieudonné, A. Beiträge zur Beurteilung der Einwirkung des Lichtes auf Bakterien. Arb. aus dem Kaiserl. Gesundheitsamt, ix, Wien., 1894, 405.

1894. Williams, E. P., and Cameron, K. Upon the general infection by the B. pyocyaneus in children. Jour. of Path. and Bact., iii, 347.

1895. Lepierre, C. Recherches sur la fonction fluorescigène des Microbes. Ann. de l'Inst. Pasteur, ix, 643.

1895. Thumm, K. Beiträge zur Kenntnis der fluorescierenden Bakt. Arb. a. d. Bakt. Inst. d. Techn. Hochschule zu Karlsruhe., i, Hyg. 2, 291.

1895. Schneider, P. Die Bedeutung der Bakterienfarbstoffe fur die Unterscheidung der Arten. Diss. Basel, 1894. Arb. d. Bakt. Inst. Karlsruhe, 1895.

1895. Schrötter, H. V. Vorlaufige Mitteilung ueber das Pigment von Sarcina aurantiaca u. Staph. pyogenes aureus. C. f. B., xvii.

1896. Beck and Schultze. Ueber die Einwirkung sogenannten monochromatischen Lichtes auf die Bakterienentwickelung. Zeitschr. f. Hyg., Bd., xxiii, 490.

1896. Biel, W. Ueber einen schwarzes Pigment bildenden Kartoffelbacillus. C. f. B., ii, Abteil, ii, No. 5, 137.

1896. Scheurlen, E. Geschichtliche und experimentelle Studien ueber den Prod. Archiv. f. Hyg , xxvi, 1.

1896. Flügge, C. Die Micro-organismen.

1897. Migula, W. System der Bakterien.

1897. Neumann, R. O. Studien u. die Variabilität der Farbstoffbildung bei M. aureus u. einigen anderen spaltpilzen. Archiv. f Hyg., xxx, 1.

1897. Noesske, H. Versuche ueber die Bedingungen der Farbstoffbildung des pyocyaneus. Beitr. z. Klin. Chirurg., xviii, 103.

1897. Radais. Sur une nouvelle race du bacille pyocyanique. Compt. rend. de la Soc. de Biol., No. 27, pp. 808–9.

1897. Wasserzug, E. Variation de forme chez les bactéries. Ann. de l'Inst. Pasteur, ii, 75, 153.

1898 Charrin et de Nittis. Sur la production simultanée des pigments, noirs, bleus, verts, jaunes, par un bacille pyocyanique. Compt. rend. de la Soc. de Biol., 1898, No. 24, p. 721.

1898. Dubois, R. Physiologie, pp. 504, 507.

1898. Heim. Bacterium pyocyaneum. Lehrbuch des Bakteriologie. Stuttgart, p. 345.

1898. Newbigen, M. I. Colour in nature, p. 28. (Good bibliography on color.)

1898. Ruzicka, Stanislav. Exper. Studien ueber die Variebilität wichtiger Charaktere des B. pyocyaneus und des B. fluorescens liquifaciens. C. f. B., I. Abteil., xxiv, 11.

1898. Niederkorn, E. Vergleichende Untersuchung ueber die verschiedenen Varietäten des Bacillus pyocyaneus u. des Bacillus fluorescens liquifaciens. Inaug. Diss. Freiburg (Schweiz), 1898. C. f. B., I., xxvii, 749.

1898. Strassburger, Noll, etc. A text-book of botany, pp. 172, 173, 201.

1899. Boland, G. W. Ueber Pyocyanin, den blauen Farbstoff des Bacillus pyocyaneus. C. f. B., xxv, 897.

1899. Jordan, E. O. Bacillus pyocyaneus and its pigments. Jour. of Ex. Med., iv, 627.

1899. Jordan, E. O. Production of fluorescent pigment by bacteria. Bot. Gaz., 1899, No. 1.

1899. v. Kuester. Versuche ueber die Farbstoff production des Bacillus pyocyaneus. Archiv. f. Klin. Chir., lx, Heft. 3, 621.

1899. Rosenberg, W. W. Beiträge zur Kenntnis der Bakterienfarbstoffe, (especially prodigiosus). Würzburg, 1899.

1899. Ruzicka, St. Bacillus pyocyaneus und Bacillus fluorescens liq. Arch. f. Hyg., xxxiv, 149; also xxxvii, 1.

1899. Serkowsky, S. Ueber die Krankheiterregende-wirkung des Bacillus pyocyaneus. Eshenedelink, 1899, No. 33, Russisch.

1899. Moynier, de Villepoix, R. Sur la presence de la bacille pyocyanique dans les cours d'alimentation. Compt. rend. de la Soc. Biol., 1899, No. 31, 828–829.

1899. Woolley, P. G. Experiment made to determine the effect of sugar upon the pigment formation of some chromogenic bacteria. Johns Hopkins Bull., x, 130, 1899.

1900. Bernhardt, R. Der Bacillus des grünen Eiters in den Harnwegen. Archiv. f. Dermatologie une Syphilis. C. f. B., I., xxviii, 601.

1900. Conn, H. W. Natural varieties of bacteria. Jour. Boston Soc. of Med. Sci., 170.

1900. Chamot, E. M., and Thiry, G. Studies on chromogenic bacteria, B. polychromogenes. Botan. Gaz., 1900, Dec., 378.

1900. Coyne et Hobbs. Appendicite à bacilles pyocyaniques. La Semaine médicale, 1900, No. 29.

1900. Helman, D. Einige Bemerkungen ueber die Rolle des B. pyocyaneus, etc. Medycyna, 1900, Nos. 47, 48, 49.

1900. Hobbs, J., and Tenier. Les essences et le pouvoir chromogène des bactéries. Ann. d'Hygiene publ. et de med. Légale, 1900, Aout, p. 103.

1900. Krause, P. Beiträge zur Kenntnis des Bacillus pyocyaneus. C. f. B., I., xxvii, 769.

1900. V. Kuester. Coloring matter of Bacillus pyocyaneus. Archiv. f. Klin. Chirurgie, lx, H. 3.

1900. Kuntze, W. Ein Beitrag zur Kenntnis der Bakterienfarbstoffbildung des B. prodigiosus. Zeitschr. f. Hyg., xxxiv, 169.

1900. Legros, G. Action sur l'organisme des pigments microbiens. La Semaine médicale, 1900, No. 46.

1900. Legros, G. Action des pigments microbiens. Compt. rend. de la Soc. de Biol., 1900, No. 33, 900.

1900. Marx, H. Eine Bemerkung zur Farbstoffproduktion der Bakterien. Archiv. f. Klin. Chir., lxii, Heft 2, 346.

1900. Matruchot, L. Sur une propriété générale des pigments bactériens. Abst gener. de botan., No. 134, 33.

1900. Noesske, H. Neue Untersuchungen ueber den B. pyocyaneus und die Gesetze der Farbstoffbildung. Arch. f. Klin. Chir., lxi, Heft. i, 266.

1900. Ritter, G. Zur Physiologie des Bacillus prodigiosus. C. f. B., II. Abteil, vi, 206.

1900. Ruzicka, St. B. pyocyaneus and B. fluorescens liq. Arch. f. Hyg., xxvii, Heft. i, 1–29.

1900. Thiry, G. N. Bacille polychrome et actinomyces mordoré; recherches biologiques sur les bactéries bleues et violettes, polychromium corps bactériens et cristaux colores, matière colorante cristallisée. (These), Nancy.

1900. Thiry, G. N. Blue pigment bacilli. "Bacille polychrome et actinomyces mordoré." These, Nancy.

1901. Christomanos, A. A. Zur Farbstoffproduktion des Bacillus pyocyaneus. Ztschr. f. Hyg., xxxvi, Heft. 2, 258–269.

1901. Gessard, C. Variété mélanogène du bacille pyocyanique. Ann. de l'Inst. Pasteur, xv, 817–831.

1901. Tiron, T. Sur les bacilles fluorescents et le pyocyanique. Jour. de Physiol. et de Pathol. gener., iii, 188–199

1901. Loew, O., u. Kozai, Y. Zur physiologie des B. pyocyaneus. Bul. College of Agric., Tokyo Imp. Univ., iv, No. 4, 227–236.

1901. Luckhardt, A. E. Ueber Variabilität in Bedingungen der Farbstoffbildung bei Spaltpilsen. Diss. Freiburg.

1902. Gessard, C. Essai sur la biologie du bacille pyocyanique. Ann. de l'Inst. Pasteur, 313.

1902. Kraft, E. Beiträge zur Biologie des B. prodigiosus u. zur Chemischenverhalten Seines Pigments. Diss. Würzburg.

1902. Krause, P. Ueber durch Pressung gewonnenen Zellsaft des B. pyocyaneus. C. f. B., I., xxxi, 673.

1902. Mazé, P. Recherches sur les modes d'utilisation du carbone ternaire par les végétaux et les microbes. Ann. de l'Inst. Pasteur, 433–451.

1902. Oliver, C. A. An experimental study of the effects of change of color upon pigment bacteria. Amer. Jour. Med. Sci., cxxiii, 647.

1902. Petrow, N. Ueber einen neuen roten Farbstoffbildenden Bacillus. Arbeit d. Bakt. Inst. d. gross. Hochschule zu Karlsruhe. Weisbaden (Otto Nemnich).

1902. Ruprecht, M. Otitis externa crouposa, durch Bacillus pyocyaneus hervorgerufen. Monatschr. f. Ohr., 512, rev., C. f. B, I., 1903, ref., xxxiii, 613.

1902. Vaughan, V. C. A study of bacterial cells. Preliminary note on certain bacterial pigments, chemistry of Sarcina lutea. Chemistry of B. coli, etc. Trans. Assoc. Amer. Phys., pp. 1–89.

1903. Chamot, E. M., et Thiry, C. Notes sur le pigment du Bacillus polychromomenes. C. R. du congrés des sociétés savantes de 1902, Paris.

1903. Bertarelli, E. Untersuchungen und Beobachtungen ueber die Biologie und Pathogenität des Bacillus prodigiosus. C. f. B., I., xxxiv, 193.

1903. Hefferan, Mary. A comparative and experimental study of bacilli producing red pigment. C. f. B., II., xi, 311.

1903. Nadson, G. Observations sur les bactéries pourprées. Bull. Jard. Imper. Bot., St. Petersbourg, iii, 1903, 99 (Russich).

1903. Papenhausen, H. Ueber die Bedingungen der Farbstoffbildung bei den Bakterien. Arb. a. d. bakteriol. Inst. d. techn. Hochschule zu Karlsruhe, iii, H. 1, 43–1000.

1903. Rubner, Max. Energieverbrauch in Leben der Mikro-organismen. Arch. f. Hyg., xlviii, 260.

1903. Samkow, S. Zur Physiologie des Bacillus prodigiosus. C. f. B., II., xi, 305.

1903. Segin, A. Ueber die Einwirkung der Bakterien auf verschiedene Zuckerarten. C. f. B., I., xxxiv, 202. Many species, sugar ferm.

1903. Sullivan, M. X. The chemistry of bacterial pigments. Science, March 6.

1903. Hargitt, C. W. Proc. Amer. Assoc. Advanc. Sci.; St. Louis.

1904. Hefferan, Mary. A comparative and experimental study of bacilli producing pigment. Diss. U. of Chicago.

Part III.

1878. Borodin, J. Ueber d. physiologische Rolle u. d. Verbreitung d. Asparagin in Pflanzenreiche. Bot. Zeit., xxxvi, 580 and 817.

1878. Schulze, E. Ueber d. Bildung v. schwefselsauren Salzen b. d. Eiweisszerzetzung in Keimpflanzen. Ber. d. deut. ch. Ges., xi, 1234.

1878. Schulze, E. Ueber Zerzetzung u. Neubildung v. Eisweisstoffen in Lupinenkeimlingen. Landwirt. Jahrb., vii, 411.

1882. Krauch, C. Ueber Peptonebildende Fermente in d. Pflanzen Landwirt Versuchs-Stat., xxvii, 383.

1883. Grimeaux, E. Synthèse der colloides azotés. C. R. de l'Acad. des Sci.. xciii, 771.

1887. Hueppe, F. Ueber Chlorophyllwirkung Chlorophyllfreier Pflanzen. Biol. C., vii, 701.

1888. Schutzenberger, P. M. Recherches sur la synthèse des matières albuminoides et protéiques. C. R. de l'Acad. des Sci., cvi, 1407.

1891. Loew, O. Die Chemischen Verhiltnisse des Bakterienlebens. C. f. B., I., ix, 722.

1891. Schutzenberger, P. M. Essai sur la synthèse des matières protéiques. C. R. de l'Acad. des Sci., cxii, 198.

1892. Griffiths, A. B. Sur la matière colorante du M. prodigiosus. C. R de l'Acad. de Sci., cxv, 321.

1894–95. Green, J. R. The reserve material of plants. Sci. Progress, ii, 109.

1895. Green, J. R. The reserve materials of plants. Sci. Progress, iii, 68, 476.

1895. Smith, T. Relation of sugars to growth of bacteria. C. f. Bakt. u. Par., xviii, No. 1.

1895–6. Brodie, T. G. The decomposition products of proteids. Sci. Prog., iv, 62.

1895–6. Halliburton, B. D. The synthesis of proteids. Sci. Prog., iv, 149.

1896. Maasen, A. Die organischen Säuren als Nährstoffe u. ihre Zersetzbarkeit d. Bakterien. Art. a. d. Kaiserl. Gesundheitsamt., xii, 340.

1896. Pammel, L. H. and Emma. A contribution on the gases produced by certain bacteria. C. f. B., xi, Zweite Abt., 633.

1897. Schulze, E. Ueber die Umsatz d. Eiweisstoffe in d. lebenden Pflanze. Z. f. Physiol., xxiv, 18.

1897. Zaleski, W. Z. Kenntniss d. Eiweissbildung in d. Pflanzen. Ber. d. deut. Bot. Gesellschaft, xv, 536.

1898. Ruppel, W. G. Zur Chem. der Tuberkelbacillus. Hoppe Seyler's Zeitschrift fur physiologische Chem., xxvi, 218.

1899. Loew, O. The physiological role of mineral nutrients. Bul. U.S. Depart. of Agri., No. 18.

1901. Levene, P. A. Bio-chemical studies on the Bacillus tuberculosis. Jour. of Med. Research, vi, 135.

1902. Rolly. Weiterer Beitrag zur Alkali und Säure produktion der Bakterien. Arch. f. Hyg., xli, 406, 1902, Heft. 4.

1902. Schreiber, K. Cleavage of fat by micro-organisms. Arch. f. Hyg., xli, 328–347.

1903. Breymann, Margarete. Ueber Stoffwechselprodukte des B. pyocyaneus. Diss. Med. Strasburg, 1903.

1903. V. Delden, A. Beitrag zur Kenntnis der Sulfatreduktion durch Bakterien. C. f. B., II., xi, 81 and 113.

1903. Grimbert, L. Diagnostic des bactéries par leurs fonctions biochimiques. Arch. de parasitol., vii, 1903, 237–305.

1903. Moll, L. Ueber künstliche Umwandlung von albumin in Globulin. Betrag z. Chem. Physiol., iv, 563.

1903. Omelianski, W. L. Ueber die Zerzetzung der Ameisensaure durch mikroben. C. f. B., II., x, 177, 256, 317.

1903. Rettger, L. F. An experimental study of the chemical products of bacillus coli communis and bacillus lactis aerogenes. Amer. Jour. of Physiol., viii, 284–293.

1903. Schittenhelm, A., et Schröter, F. Gasbildung und Gasathmung von Bakterien. C. f. B., xxxv, 146.

1903. Schittenhelm, A., et Schröter, F. Ueber die Spaltung der Hefnucleinsaure durch Bakterien. Zeit. f. Phys. Chem., xl, 1903, 62, 69, 80.

1904. Schroeder, Max. Beiträge zur Kenntnis der Stoffwechselprodukte des Bac. lact. aërogenes. Aus. dem. hyg. Inst. der Univ. Strassburg, C. f. B., II., xi, 732.

1904. Stalstrom, A. Beiträge zur Kenntnis der Einwirkung steriler und in Garung befindlicher organischer Stoffe auf die Löslichkeit der Phosphorsäure des Tricalcium phosphate. C. f. B., II., xi, 724.

1905. Folin, Otto. A theory of protein metabolism. Amer. Jour. of Physiol., xiii, No. 11.

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[FROM THE LABORATORY OF THE U. S. BUREAU OF FISHERIES, WOODS HOLE, MASS., AND THE LABORATORY OF COMPARATIVE ANATOMY, BROWN UNIVERSITY.]

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SIXTEEN per cent of the dogfish taken at the laboratory of the Bureau of Fisheries, Woods Hole, Mass., during the summer of 1904, were found to contain lobsters; 34.17 per cent rock crabs, and 20.1 per cent spider crabs. The carapace of these organisms consists of salts and chitin, which is highly resistant to reagents. As the carapace was found in varying degrees of decomposition, and further since the carapace of crabs and lobsters fed to the fish could not be found in the stomach after four days of digestion, the question arose as to whether the dogfish really did digest chitin. Accordingly, during this summer I began a physiological study of the alimentary canal of Mustelus canis, and extended the work to include the sand shark (Carcharias littoralis) and the spiny dogfish (Squalus acanthias).

The experiments of 1904 consisted in the study of the normal contents of the stomach and the spiral valve; in the determination of the digestive power of the juices contained in the stomach and spiral valve; in the preparation for artificial digestion of extracts of buccal, esophageal, gastric, and intestinal mucous membranes, and of the extracts of the pancreas; and finally in determining whether or not these fishes digested chitin.

During the summer of 1905 the work of the previous summer was reviewed, amplified, and extended to include the torpedo (Tetronarce occidentalis), the dusky shark (Carcharhinus obscurus), the skate (Raja erinacea), and the mackerel shark (Lamna cornubica). More attention was paid to the physiology of the pancreas, middle intestine, and spleen; and histological preparations were made of the various parts of the alimentary canal from the esophagus to the anus. The résumé of the work is as follows:

(A) **Buccal cavity.** — These fish as a rule swallow their prey whole without mastication. Naturally, we should suppose that the buccal mucus has little digestive action. This probability is increased by the absence of glands. The buccal mucus consists of epithelium and connective tissue. There are several layers of epithelium. Next to the connective tissue are cylindrical cells. Above these cells are several layers of mucous cells. The superficial epithelium consists of oval or irregularly polygonal cells. Extracts of buccal mucus have no digestive action on starch, fibrin, or fat.

(B) **Esophagus.** — The reaction of the esophagus is often acid, probably due to regurgitation from the stomach. Extracts of esophageal mucus of the fasting fish have no digestive action. The mucus contains ciliated cylindrical cells and goblet cells.

(C) **Stomach.** — The stomach of elasmobranchs consists of a large cardiac sac and a narrow pyloric tube.

Cardiac sac. — An analysis of the contents of the sac of Mustelus canis, Carcharias littoralis, Squalus acanthias, Tetronarce occidentalis, Carcharhinus obscurus, Raja erinacea, Lamna cornubica, Galeocerdo tigrinus, showed as a rule syntonin, proteoses, and peptones. Occasionally in the stomach contents no peptones could be found. Using phenolphthalein, alizarin, and dimethyl-amido-azobenzol, as indicators, experiments were made to determine —

1. The total acidity of the stomach contents in terms of hydrochloric acid.
2. The physiologically active hydrochloric acid.
3. The free hydrochloric acid.

The results are given in the table on page 44.

The acidity of the stomach contents depends on the period of digestion, the fasting stomach being practically neutral, and upon the nature of the food, the greatest acidity being found when the stomach was full of partly digested lobsters and crabs.

Glycerin-hydrochloric acid extracts of the mucus of the cardiac sac digest fibrin at 20° C., but better at 38° C., with the formation of syntonin, proteoses, and peptones.

In the cardiac part of the stomach we can distinguish superficial epithelium, the lumen of the peptic glands, and the glandular epithelium in the crypts. The superficial epithelium consists of pyramidal cells. The peptic glands begin just behind the esophagus and extend to the pylorus, with the deepest glands in the centre. Each gland is a cylindrical tube. The epithelium of the neck of the glands consists of cylindrical cells, while the cells of the body of the glands are regularly polygonal, highly granular, and closely packed together. Only this one kind of cell can be distinguished in the body of the gland.

Pyloric tube. — The pyloric tube has no digestive function. The superficial epithelium is like that of the stomach. The glands are short, and the polygonal peptic cells are absent.

(D) **Middle intestine.** — Extracts of the middle intestine or duodenum of Mustelus canis and Carcharias littoralis show no digestive action. The epithelium consists of cylindrical cells and goblet cells.

Species.	Total acidity in percentage hydrochloric acid.	Physiologically active hydrochloric acid, average percentage.	Highest percentage free hydrochloric acid.
Mustelus canis	0.04–1.00 Aver. 0.73 50 individuals	0.538 6 individuals	0.2
Carcharias littoralis . . .	0.1–1.2 Aver. 0.87 25 individuals	0.614 10 individuals	0.31
Squalus acanthias	Aver. 0.67 60 individuals	No tests	No tests.
Carcharinus obscurus . .	Aver. 0.55 2 individuals	0.493 2 individuals	0.254
Lamna cornubica	0.275 1 individual	0.229 1 individual	0.172
Galeocerdo tigrinus . . .	0.93 1 individual	0.812 1 individual	None.
Tetronarce occidentalis . .	0.51 1 individual	No tests	None.

(E) **Spiral valve.** — The arrangement histologically is like that of the middle intestine. Extracts of the spiral valve show no digestive action.

(F) **Pancreas.** — Extracts of the pancreas vary in their action. Some have no digestive action whatever. Neither water extracts nor sodium carbonate extracts of the pancreas of the various elasmobranchs have any digestive action on hard-boiled egg or fibrin. Extracts of the middle intestines do not activate the pancreas. Bile activates the pancreatic extracts slightly. The spleen activates the pancreas most. A boric acid extract of the pancreas, plus a boric extract of the spleen of a fish in full digestion, gave the greatest digestion of fibrin. Pancreatic fistulas were of little value. Of six made, but one gave a juice having any digestive action on fibrin, and even this was slight.

Analysis of the contents of the spiral valve showed leucin and tyrosin, proving that the pancreas acts in these animals as in the higher animals. The fresh pancreas emulsifies olive oil. Glycerin-acetic extracts of Mustelus canis convert starch to sugar. None of the standard extracts of the pancreas of Carcharias littoralis and Raja erinacea showed the amylolytic ferment.

(G) **Rectal gland.** — The rectal gland is a compound tubular gland. Extracts of this gland do not show any digestive activity.

(H) **Action of gastric juice on chitin.** — The natural juice of the stomach of Mustelus canis digested fish and fibrin in vitro with the formation of syntonin, proteoses, and peptones, but did not digest the carapace of lobsters and crabs. The frequent change of the gastric juice and much trituration, however, softened the shells and broke them up into a fine mass such as may be found in the spiral valve of the fish. The chitin is not regurgitated. Pieces may be found intact in the spiral valve. These fishes do not derive nourishment from the carapace, but dissolve out the salts by means of the relatively strong acid which collects in the stomach, and triturate the chitinous mass into extremely fine particles. This finely divided mass is excreted.

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